Clean Water Act Section 319(h) Nonpoint Source Pollution

Control Program Projects

Upper Colorado Salt Cedar Control Project:

Biological Control Component -

Quality Assurance Project Plan

Prepared by

Texas State Soil & Water Conservation Board

and

United States Department of Agriculture

Agricultural Research Service

Effective Period: 3 Years

24 October 2003 to 31 March 2006

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Section A1: Approval Sheet

Quality Assurance Project Plan for Project. Upper Colorado River (Texas) Saltcedar Control Project: Biological Control Component – 1st Revision

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Name: Donna Long
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Signature: Donna K. Long Date: 22 Jan 2007

U.S. Department of Agriculture, Agricultural Research Service

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Title: ARS Project Manager/QA Manager
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Title: *Acting* Laboratory Director
Signature: H. Wayne Polley Date: 19 Jan. 2007

Section A2: Table of Contents

Section	Title	Page
A1	Approval Sheet	2
A2	Table of Contents	3
A3	Distribution List	6
A4	Project/Task Organization	7
A5	Problem Definition/Background	10
A6	Project Description	24
A7	Data Quality Objectives and Criteria for Measurement Data	32
A8	Special Training Requirements/Certification	34
A9	Documentation and Records	35
B1	Sampling Process Design (Experimental Design)	36
B2	Sampling Methods	41
B3	Sample Handling and Custody Requirements	49
B4	Identification of Insect and Plant Samples	50
B5	Quality Control Requirements	51
B6	Equipment Testing, Inspection, & Maintenance Requirements	53
B7	Instrument Calibration and Frequency	54
B8	Inspection/Acceptance Requirements for Supplies and Consumables	55
B9	Data Acquisition Requirements (Non-direct Measurement)	56
B10	Data Management	57
C1	Assessments and Response Actions	58
C2	Reports to Management	59
D1	Data Review, Validation, and Verification	60
D2	Validation and Verification Methods	61
D3	Reconciliation with Data Quality Objectives	62
D4	Information Dissemination	63
Appendix A	Forms Used	64-73

List of Tables

A5-1	Multiple-choice host selection test by larval and adult *D.e. deserticola* from Fukang, China and Chilik, Kazakhstan, 2000, at Temple, TX	18
A5-2	Host selection by adult *Diorhabda elongata*: Paired-choice Adult tests, Temple, TX, July 2003	20
A5-3	Ovipositional host selection by female *Diorhabda elongata*: Paired-choice adult tests, Temple, TX, July 2003	20
A5-4	Field releases of new *Diorhabda elongata* biotypes in southern areas in 2003	23
A6-1	Project plan milestones	31
B1-1	Sampling sites locations monitored by ARS	36
B1-2	Biological, Ecological and Control Monitoring Breakdown	40

List of Figures

A4-1	Project Organization Chart	9

List of Acronyms and Abbreviations

ARS – Agricultural Research Service (USDA)
BC/SC – Biological Control of Saltcedar
CRMWD – Colorado River Municipal Water District
GSWRL – Grassland, Soil and Water Research Laboratory
SEL – Systematic Entomological Laboratory (USDA-ARS)
TAES – Texas Agricultural Extension Service
TAMU – Texas A&M University
TSSWCB – Texas State Soil and Water Conservation Board
USDA – United States Department of Agriculture

Section A3: Distribution List

Organizations, and individuals within, which will receive copies of the approved QAPP and any subsequent revisions include:

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Section A4: Project/Task Organization

The following is a list of individuals and organizations participating in the project with their specific roles and responsibilities:

USEPA – United States Environmental Protection Agency (USEPA), Region VI, Dallas. Provides project overview at the Federal level.

Ellen Caldwell, USEPA Texas Nonpoint Source Project Manager
Responsible for overall performance and direction of the project at the Federal level.
Approves the final products and deliverables.

TSSWCB – Texas State Soil and Water Conservation Board (TSSWCB), Temple, Texas.
Project Lead.
Laurie Fleet, TSSWCB Project Manager
Donna Long, TSSWCB Quality Assurance Officer

Project Implementation Personnel

C. Jack DeLoach, Ph.D. – Project Lead (ARS) – Plans research and field demonstrations. Oversees the research execution and data collection, supervises personnel, authorizes expenditures, writes reports to TSSWCB, maintains the official, approved Project Research Plan.

C. Jack DeLoach, Ph.D. – Quality Assurance Manager (ARS) – Prepares QAPP and forwards to TSSCWB for approval. Assures that experiments and demonstrations are conducted and data is collected according to approved QAPP. Maintains the official, approved QA Project Plan.

Allen Knutson, Ph.D. – Entomologist, TAES, Dallas – Conducts independent research on dispersal of *Diorhabda* beetles and effects of beetle defoliation on carbohydrate reserves of saltcedar plants; cooperates with ARS in releases and monitoring of *Diorhabda* beetles.

Lindsey Milbrath, Ph.D. (Post Doctoral Scientist) – Project Biologist – Conducts research and field demonstrations on host specificity, field ecology and field biology of the *Diorhabda* beetles, cooperates with research projects of TAES scientist Dr. Allen Knuston.

James Tracy, M.S. – ARS Project Lead Technician – Sets up experiments and demonstrations in the field, performs monitoring of *Diorhabda* beetles, vegetation and butterflies described in Research Plan; supervises daily work activities and is assisted by temporary personnel.

Tom Robbins, B.S. – ARS Project Technician – Works together with Technician Tracy in collecting data on monitoring of *Diorhabda* beetles and vegetation surveys. Conducts and coordinates bird monitoring with temporary ornithologist Johnson. Is the primary identifier of plants and insects discovered during the monitoring.

Jeremy Hudgeons, M.S. Graduate Assistant of Dr. Knutson, Texas A&M University, Department of Entomology.

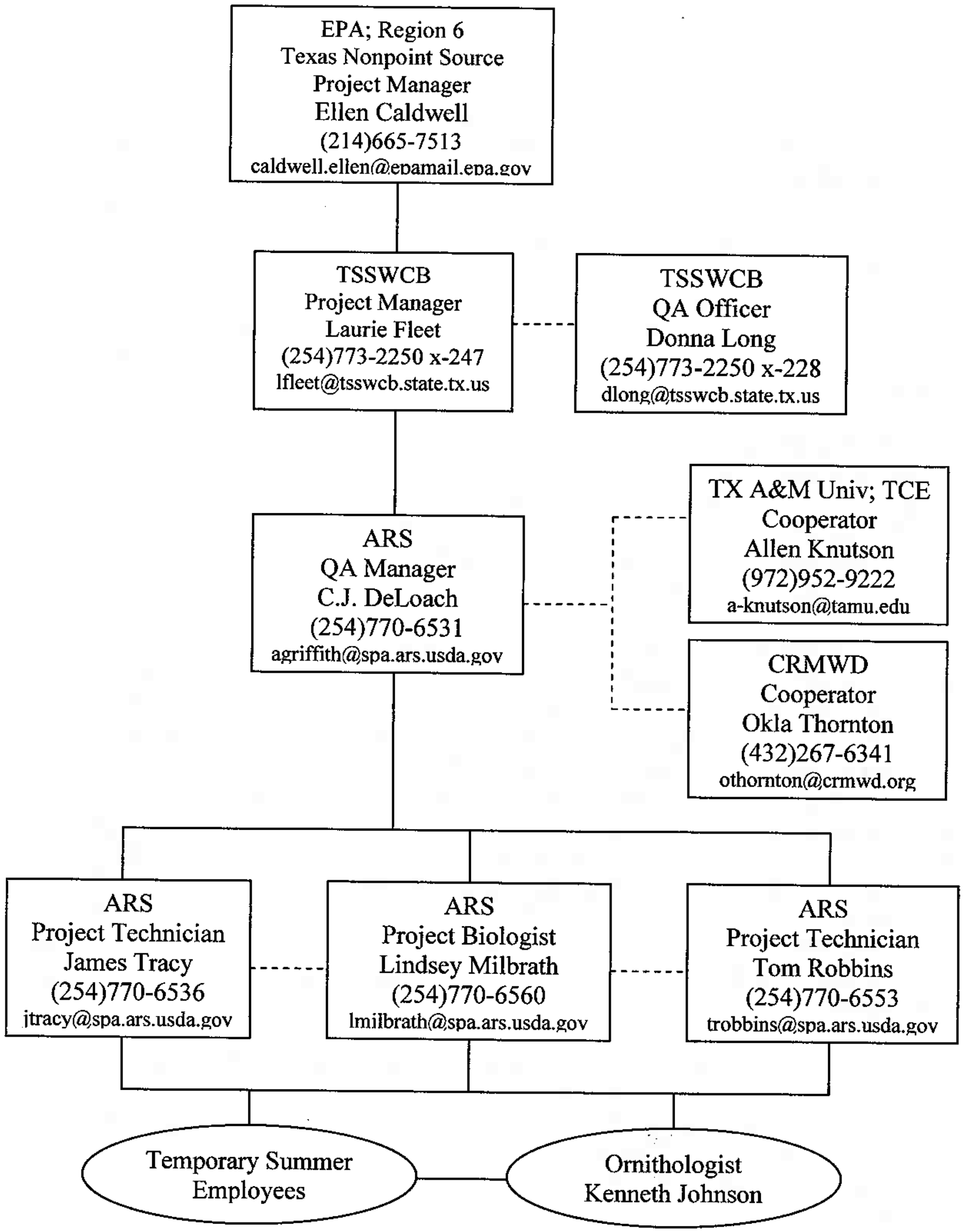
Okla Thornton, M.S. – Ecologist, Colorado River Municipal Water District(CRMWD) – Assists in site location and planning of beetle, plant and wildlife monitoring; liaison with local landowners and water district operations.

Kenneth Johnson, Ph.D. – Ornithologist, biologist – Together with Technician Robbins conducts bird monitoring.

Principal Data Users

Ranching community (ranchers, landowners)
USDA (ARS, APHIS, FS, NRCS)
USDI (BR, BLM, NPS, FWS, BIA)
USDOD (COE, Army, Air Force, Marines)
State Agencies: Texas: Department of Agriculture, Parks and Wildlife Department, TSSWCB, TX Riparian Invasive Plant Task Force, TX Agricultural Extension Service, TX A&M University; NM: Department of Agriculture, NM State University
Water users: agricultural, residential, municipal, industrial, environmental; Colorado River Municipal Water District

Fig. A4-1. Project Organization Chart



Section A5: Problem Definition/Background

A. Need for control of saltcedar

The invasion by exotic saltcedars, small trees or shrubs from Eurasia, along western U.S. streams and lakeshores has produced one of the worst ecological disasters in the recorded history of the region. The plant was first recorded in a plant nursery in 1823, and thereafter it was widely planted throughout the West as an ornamental and to control streambank erosion. It had escaped cultivation by the 1890's, was noted as a pest in some areas by 1910, it rapidly invaded riparian areas after the late 1920s, and by 1950 it occupied large areas of many western riverbottoms and lakeshores (Robinson 1965). Today it is still spreading along tributaries and small streams. Worldwide, 54 species are recognized, with the centers of origin from central Asia to China and in the eastern Mediterranean area (Baum 1978). Some 10 species have been introduced into the U.S.; 4 of them, and their hybrids (Gaskin and Schaal 2002), cause almost all of the damage (reviewed by DeLoach et al. 2000, 2003).

1. <u>Environmental Damage</u>
Dense thickets of saltcedar have displaced the native plant communities. Saltcedars are heavy water users, lower water tables and cause small streams and desert springs to dry up, increase soil salinity and wildfire frequency, and reduce recreational usage of parks and natural areas. They alter stream channel structure, cause bank aggradation, narrowing, deepening and blockage of channels, and alter water quality.

These changes to the plant community and to the physical environment combine to severely degrade wildlife habitat. The native wildlife (mammals, birds, reptiles and amphibians, fishes, insects and other invertebrate) have not evolved with saltcedar and are largely unable to utilize it or to adapt to the environmental changes it produces. Saltcedar foliage is rather unpalatable, its tiny fruits and seeds are not utilized, cavity dwellers and granivores are mostly absent in saltcedar thickets, most native insects are unable to develop on it though many are attracted to its flowers, and the altered aquatic environment is harmful to many fish, amphibians, and to the species of insects and invertebrates on which they feed. Saltcedar has greatly reduced biodiversity in the majority of the vital southwestern riparian ecosystems. Many wildlife species have declined as saltcedar has replaced the native plants, several have become endangered, and at least 50 T&E species, mostly fishes and birds but including also mammals, reptiles, insects and other invertebrate and plants have been severely affected (reviewed by DeLoach and Tracy 1997, DeLoach et al. 2000).

The southwestern willow flycatcher *Empidonax trailii* subspecies *extimus* (sw WIFL), was placed on the Federal endangered species list in March 1995. This small, neotropical-migrant, mid-summer breeding, riparian obligate bird breeds in southern California, most of Arizona, eastward to the Rio Grande in New Mexico, in southwestern Colorado, in southern Utah and Nevada, and historically along the Rio Grande of westernmost Texas. Today, it does not occur east of the Rio Grande of central New Mexico or anywhere in Texas.

The interactions between the sw WIFL and its habitat was reviewed by Finch and Stoleson (2000), and especially between it and saltcedar by DeLoach and Tracy (1997), DeLoach et al. (2000), and Dudley et al. (2000). Its populations have declined precipitously in recent years, in close correlation with the decline in its native willow-cottonwood riparian habitat and the increase of saltcedar. However, in mid-elevational areas of Arizona (but not in other states) it nests extensively in saltcedar in areas where saltcedar has replaced the native trees. It chooses saltcedar nest trees even if apparently suitable willows are abundant nearby. This appears to be a case of the classical ecological concept of a "super normal stimulus" in which one stimulus (in this case the near ideal branching structure of saltcedar for nest placement) overrides all other stimuli even if such selection overall is detrimental to the bird. Nearly all known or suspected mortality factors of the sw WIFL are made worse by saltcedar, including loss of habitat, cowbird predation, need for free water in streams, lakes or flooded areas, lack of proper food (insect larvae), lethal high temperatures, and possibly stress on the females. This results in a reproductive success in saltcedar of only half that in cottonwood/willow dominated habitats (DeLoach and Tracy 1997, DeLoach et al. 2000, DeLoach et al., MS submitted 2000). However, substantial population increases recently have been reported as willows have revegetated, as along the middle Rio Grande of New Mexico and at Roosevelt Lake, Arizona.

A major concern stated by flycatcher biologists is that in many areas now occupied by saltcedar the water tables are too low and the soil salinity too high to allow revegetation by cottonwoods and willows after saltcedar control and the sw WIFL would loose its breeding habitat. This would be a concern only in Arizona because in other states the sw WIFL breeds only or mostly in native habitat. Also, in all the major sw WIFL breeding areas, both depth to water table and salinity levels are suitable for cottonwoods and willows, as evidenced by their presence; their low abundance is probably because of competition from saltcedar. Surveys by the Bureau of Reclamation (USDI-BOR 1995) demonstrated that along the lower Colorado River downstream from Lake Mead most of the potential breeding area is suitable for cottonwood/willow, including all of the major breeding area at Topock Marsh. The complete lack of breeding in this major area of former breeding south of Topock Marsh is probably caused by the saltcedar invasion and that temperatures for the willow thickets often exceed the lethal high temperature for survival of bird eggs, whereas the former upper canopy of tall cottonwoods and understory of willows was cooler. Several areas along the river have revegetated naturally with cottonwoods and willows since the El Niño floods of the mid 1980s and mid 1990s (DeLoach et al. 2000; manuscript submitted 2002).

Major revegetation experiments are underway by the Bureau of Reclamation to develop methodologies for restoring the native vegetation. Large projects are in progress at San Marcial on the Rio Grande and are planned for Lake Merideth and Big Bend National Park, TX and along the Lower Colorado River, CA/AZ (Ken Lair and Sarah Wynn, BOR, Denver). At recent manual revegetation sites along the lower Colorado, the transplanted cottonwood and willow poles are growing beautifully and rapidly (DeLoach and Sarah Wynn, personal observations, 2001).

2. Depletion of Water Resources for Agriculture and Municipalities

Numerous large-scale experiments measured water usage by saltcedar from the 1940s to the 1980s, along the Gila River, NM (Gatewood et al. 1950; Culler et al. 1970, 1982), the middle Rio Grande (Bureau of Reclamation 1972, 1973; van Hylckama 1968, 1974, 1980; Gay and Fritschen 1979), the lower Colorado near Blythe, CA (Gay and Samis 1977, Gay and Hartman 1982, Gay 1985), and along the Pecos River near Artesia, NM (Weeks et al. 1987). Usage was greatly influenced by depth to water table, water salinity, density and size of the plants, growth stage of the plant, season of the year (temperature/daylength), and latitude/elevation above sea level (also temperature/daylength). Summaries of this research by Johns (1989), Horton (in Brown et al. 1989) and DeLoach (1991) indicated that water usage by saltcedar varied from 3 ft/yr at Bernardo, NM to an average 5.7 ft/yr at Blythe, CA.

At Artesia, NM from 1980 to 1982, old growth saltcedar (10 ft water table) used 2.75 mm/day, wet old growth (2-3 ft water table) used 5.2 mm/day, burned in 1974 (4-6 ft water table) 3.65 mm/day, and mowed in 1977 (10 ft water table) used 4.87 mm/day. Average usage in all plots was 35.4 (30.1 to 42.1) inches/year and replacement vegetation (grass and forbs) used 22.4 to 26.4 in/year, giving a calculated salvage of 11.0 in/year by the energy-budget method or 7.9 in/yr by the eddy correlation method (Weeks et al. 1987).

Below average rainfall over the past decade, together with saltcedar depletion of stream flow (estimated at one-third of the total allowable annual depletion of riverflow, has created urgent water shortages for agriculture and municipalities throughout the southwest. This has resulted in default of water agreements between states and between the United States and Mexico, with serious economic and political consequences. Large-scale and expensive saltcedar Aeradication@ programs have been initiated by the Departments of Agriculture of Texas and New Mexico, by many affected water districts, and as proposed for Federal funding in these and other western and southwestern states.

Along the Rio Grande, one-third of the allowable annual depletion of water is lost to saltcedar (Steve Hansen, Bureau of Reclamation, Albuquerque, personal communication). Water used by saltcedar, above and beyond that used by the native vegetation, is estimated to be sufficient to supply the needs of 20 million people (Tim Carter, personal communication). The present severe drought has reduced the streamflow available for irrigated agriculture and municipal use, threatening the livelihood of farmers, causing water rationing in towns and cities. Flow from the Rio Grande no longer reaches the Gulf of Mexico.

Some studies also showed that water usage by native phreatophytes, especially by cottonwoods and willows (the most valuable wildlife habitat) was equal to saltcedar (reviewed by DeLoach 1991). However, the studies did not consider that saltcedar, because it is a deep-rooted facultative phreatophyte, can take water from much deeper in the soil, and can occupy an area much further from the streambanks or lakeshores, and thus occupy a much larger area of the valley and can consume much more water on a river-valley basis than can willows and cottonwoods (Smith et al. 1998).

3. Causes of the Saltcedar Invasion

The invasion of saltcedar is thought by many to be caused mostly by abiotic or human produced environmental changes B dam building, livestock grazing, groundwater pumping, etc. B and that the invasion was only passive and followed these changes (Everitt 1998, reviewed by DeLoach et al. 2000). Its innate aggressive characteristics appear to make its invasion unstoppable and its domination of ecosystems to appear invincible. Saltcedar appears to be more aggressive and better adapted to the changed environment than are the native plant communities it has replaced. Saltcedar qualifies under 10 of the 12 criteria that Baker (1974) used to characterize the ideal weed.

However, saltcedar also has invaded small streams and desert springs far removed from altered river hydrologic cycles, livestock, or other obvious human influence (Lovich and deGouvenain 1998, Barrows 1998). Its invasion also is promoted by several important biotic factors that are little recognized B its direct competition with the native plants for water, nutrients, light (Smith et al. 1998); its synergistic interactions with the abiotic/anthropogenic factors; its alteration of the physical environment (increased soil salinity and wildfires and decreased water availability); and very importantly, the lack of natural enemies (insects, plant pathogens) that damage it (DeLoach et al. 2000).

The unique ecological and physiological characteristics of saltcears allow it to interest synergistically with many natural factors or human ecosystem modifications in a feed-forward manner to increase its own competitive advantage over the native plant communities. The construction of dams alters the natural flood cycle to exclude spring germination of cottonwood/willow seeds but to allow summer germination of saltcedar seeds, saltcedar lowers water tables below the root level of the native cottonwoods and willows, it increases wildfires and soil salinity to which it is tolerant but which kill the natives, it is more tolerant of livestock browsing than are the natives, and herbicide or mechanical controls used to control it also kill many native plants. Importantly, the native insects and plant pathogens that constantly suppress native plant communities but they do not damage saltcedars (DeLoach et al. 2000).

4. Conventional Control of Saltcedar

Saltcedar, during the past 50 years, has proven to be a difficult and expensive invasive weed to control. They propagate both by huge numbers of tiny windblown seeds and vegetatively, they are facultative phreatophytes and halophytes, and they are tolerant of fire, drought and inundation. Programs to control saltcedar (and native phreatophytes as well) have been conducted several times in the past, most notably during and after the drought of the 1950s (PSIAC 1966, Pinkney 1990, Sisneros 1990; reviewed by DeLoach 1989, DeLoach and Tracy 1997), but the effect always has been short lived because of regrowth and reinvasion. The present drought makes rapid control urgent.

Large-scale herbicidal and mechanical control programs are in progress along the Pecos Rivers of Texas and New Mexico and are planned to include the Rio Grande, and the Colorado, Brazos, Frio, and other infested rivers and their tributaries in western Texas. Similar programs may be initiated in several other western states. These treatments primarily use Arsenal and Rodeo applied by helicopters. In areas of present monotypic saltcedar stands (especially prevalent along the saline Pecos River) these controls are expected to provide rapid control and immediate water salvage, and with little or no detrimental side effects, though several years will be required to treat all areas (Hart et al. 2000).

5. Appropriateness of Biological Control for Saltcedar (BC/SC)

a. Biological Control of Weeds in General. Biological control is highly specific, killing only one or a few closely related plants. It is most useful in natural areas, rangelands and forests, where the ideal objective is to kill only the target weed and leave unharmed all the other plants, the opposite of the objective for herbicides in cultivated crops.

Three approaches to biological control are usually recognized. In "Conservation", the methodology is to develop techniques that conserve the natural enemies that control the target pest. In "Augmentation", methods are developed for increasing the numbers of control agents, such as by mass rearing and release. The "Classical" or "Introductory" approach for weed control is to introduce the highly host specific natural enemies (usually insects or plant pathogens) that suppress the weed's populations in its homeland. The philosophy, methodologies, and safety guidelines and regulations have been well developed especially since the late 1950s (Huffaker 1957, and as reviewed by DeLoach 1997). Today, they offer highly accurate methods for determining the safety of candidate control agents, but less accurate methods for predicting degree of control after release. Historically, this approach has been by far the most often used and the most successful (Julian and Griffiths 1999). The classical approach is relatively inexpensive, permanent, highly host specific, and environmentally compatible. The objective is not to eradicate the weed (which biological control has never done) but to reduce the abundance below the level where economic or ecological damage occurs.

Biological control kills the target weeds even in mixed stands without harming other plants, the control agents actively seek out the target weed even in areas of difficult access, and it provides permanent suppression of the target weed so that reinfestation does not occur (therefore, 100% control to eliminate weed reservoirs of reinfestation is unnecessary). It does not contain chemicals that pollute the environment, and it is relatively inexpensive because every plant in the infested area does not need treatment and repeated applications are unnecessary. During the history of biological control of weeds, no damage has been reported to non-target plants except for 8 cases of minor damage during the 1960s, most of them of short duration, that would not occur under present guidelines and regulations. All cases of non-target feeding, including that of the well-known seed-head fly that controls must thistle, were predicted in the pre-release testing. No case of a control agent changing its host range is known (McFadyen 1998, Marohasy 1996).

Disadvantages of biological control are that the control agents, once released, cannot be limited to certain areas, control may be somewhat slow, requiring a few years to achieve satisfactory control level in a given area and several years to spread to other areas unless redistributed manually. Suitable control agents sometimes cannot be found that have narrow host ranges and also provide control in all climatic zones or in all habitats. Sometimes, naturally occurring parasites and predators limit the effectiveness and too-frequent applications of herbicides can prevent the control agents from reaching controlling levels.

Classical biological control has been used against 130 weed species in 51 countries, and using 272 introduced control agents since 1865. Control agents have been released to control 40 exotic weed species in the continental United States and Canada since 1945, and against 25 exotic weed species in Hawaii since 1902. About one-third of these weeds have been successfully and permanently controlled, with great benefit to natural areas and to agriculture. Another third have been partially controlled and a third with little or no control; many of the latter have received little research effort or are new projects. Greatest effectiveness often is obtained by introducing control agents that attack different parts of the weed, such as foliage feeders, seed feeders, stem or root borers, etc. (Julien and Griffiths 1999). In the continental United States, successful control has been obtained of St. Johnswort, puncturevine, tansy ragwart, muskthistle, alligatorweed, waterhyacinth, waterlettuce, skeletonweed, field bindweed, leafy spurge, and purple loosestrife (Nechols et al. 1995, Rees et al. 1996). Several other projects appear to be nearing success, such as melaleuca, giant salvinia, Old World climbing fern, Brazilian pepper tree, yellow starthistle, houndstongue, toadflax, some knapweeds, and, hopefully, saltcedar.

The protocol for the "introductory" approach is to 1) find and select the best of the highly host specific insects or plant pathogens that damage the weed (those that cannot complete their life cycle on other plants) within the weed's native distribution in other countries (Goeden and Harris 1982), 2) determine the control agent's biology, ecology and host range, 3) introduce them into quarantine in the United States for final host range and biological testing and to produce "clean" colonies free of predators, parasitoids or pathogens; 4) after obtaining the proper authorizations, to release them into the field; and 5) monitor the control obtained and the effects produced in the natural and agricultural ecosystems.

The methodologies of biological control of weeds, including host-range determination of the control agents, have been developed to a high state of reliability over many years (Huffaker 1957, Harley and Forno 1992, Rees et al. 1996, DeLoach 1997). A variety of tests are used depending on the life history of the control agent, such as adult or larval feeding, either no-choice or multiple-choice, or ovipositional host selection (Huffaker 1964, Harris and Zwölfer 1968, Zwölfer and Harris 1971). Test plants for host specificity testing are selected by the centrifugal-phylogenetic method whereby plants most closely related to the target weed (same genus) are tested first; if feeding occurs on other species, then species of other genera (same family) are tested, and so on until the host range is defined or the test insect is shown to have too broad a host range to be introduced (Zwölfer and Harris 1968, Wapshere 1974). Since no species of the family Tamaricaceae are native or are beneficial exotics [except for the exotic athel (*Tamarix aphylla*)] in North America, a control insect would be acceptable for introduction so long as it does not complete its life cycle on species outside the Tamaricaceae and does not cause great damage to athel, and does not damage the native *Frankenia* spp.

b. Biological Control of Saltcedar (BC/SC). Saltcedar ranks very high under nearly all of the characteristics generally accepted as qualifiers for biological control: it is an exotic invader, it is not closely related to any native or economically important plants in North America, it causes great losses and has small beneficial values, it occurs in stable ecosystems, and many promising control agents are known in its native range that are highly specific and potentially could be introduced (DeLoach 1989, 1991, 1996; DeLoach and Tracy 1997).

Biological control offers the potential for effective control of saltcedar. It is highly specific to saltcedar and can control only it in mixed stands without damage to any other plants. It also is relatively inexpensive and provides permanent control, including control of regrowth and of reinfestations. Although it will not eradicate saltcedar (nor will any other type of control), the 75 to 85% control expected (which could reach 95% control in some areas) is sufficient to greatly reduce water losses; to allow recovery of native vegetation, wildlife, and fishes; to reduce wildfires and salinization of soils; and to allow satisfactory recreational usage of riparian areas. The potential for successful control is great based on the large number of host-specific insects known to attack saltcedar in the Old World and on early field test results with leaf beetle, *Diorhabda elongata*.

The major concern in the use of biological agents to control saltcedar is for the possible loss of habitat for the endangered southwestern willow flycatcher (sw WIFL) that has begun nesting in saltcedar in mid-elevational areas of Arizona and southernmost Nevada in recent years, since its native willow nest trees have been replaced by saltcedar (DeLoach and Tracy 1997, DeLoach et al. 2000). This was the main topic addressed by the Biological Assessment submitted to the U.S. Fish and Wildlife Service in October 1997 (DeLoach and Tracy 1997) and of the Research Proposal of 28 October 1998 (DeLoach and Gould 1998). However, the Biological Assessment (and DeLoach et al. 2000) concluded that biological control is unlikely to adversely affect the sw WIFL or any other of the 51 endangered or threatened species that occur in or near saltcedar infested areas of the United States.

Any possible effects of biological control on the sw WIFL is not expected to be a factor in the Upper Colorado, TX saltcedar control project. The flycatcher does not and never has occurred within the control area, the nearest sw WIFL breeding area (only a few nests in saltcedar stands) are at Elephant Butte Lake State Park and at the Sevilleta NWR, on the middle Rio Grande, NM, more than 200 miles to the west, and with no streams that connect the Rio Grande and the Colorado River of Texas.

The principal disadvantage of biological control is that 3 to 5 years probably would be required for it to achieve its potential in an area of a mile radius around a release site. However, control could be obtained throughout Sector 1 of the project (if the beetles are as effective as indicated in recent field tests) if they are redistributed manually. Such releases are inexpensive once a large population of beetles is established at one location in the field and are available for redistribution. The degree of control that will be produced by the *D. elongata* beetles along the Colorado River is still somewhat uncertain. Both the physical and the biotic environmental factors vary between locations and their effect on the beetles cannot be fully predicted before release. Two years after the release of *D. elongata* into the open field at the 6 most northern sites in Colorado, Wyoming, Utah, Nevada and California, where it is adapted to the long summer daylength, *D. elongata* attained high populations at 5 sites and it has produced severe defoliation of saltcedar at 2 sites. These results (and those of several other successful biological control of weeds projects) indicate that biological control is potentially capable of controlling saltcedar in all situations from monotypic stands of large trees to dispersed or mixed stands of large or small trees. In several other successful projects, biological control was the only control used and herbicidal or mechanical controls were unnecessary.

In situations of acute water shortage such as exist in Texas and the other Southwestern states where rapid control is essential, biological control is the method of choice a) to follow herbicidal treatments to control regrowth and reintroductions of saltcedar, b) to use in areas of mixed native/saltcedar vegetation where protection of the native plants is important and where the hand application of herbicides that would be required to protect the native plants is prohibitively expensive, c) for use in areas where herbicides are unlikely to be used over the next 3 or 4 years, and d) to obtain long-term and permanent control. Once the initial dense saltcedar stands have been reduced by herbicides and the biological control insects have become established, further herbicidal control may be unnecessary. In fact, the continued frequent use of herbicides is likely to prevent permanent, effective biological control by reducing the food supply of the control insects so that they cannot maintain controlling populations to provide continuing control of regrowth and reinvasion.

B. Previous research on biological control of saltcedar (BC/SC)

Biological control of saltcedar was begun by USDA-ARS at Albany, CA in the 1970s with explorations for candidate natural enemies in Israel, Italy, Turkey and Pakistan. This research and that of scientists in the Soviet Union, revealed over 300 insect species in Asia, with several also in southern Europe and northern Africa, that damage saltcedar but that apparently do not attach other plants. Research toward testing and release of natural enemies was begun by USDA-ARS at Temple, TX in 1987, joined by USDA-ARS at Albany, CA in 1998. Some 20 species are undergoing preliminary testing by overseas cooperators in Kazakhstan, China, Israel and France and some 10 species are being tested in quarantine at Temple and Albany (DeLoach 1989, 1990; DeLoach et al. 1996). Three species have received TAG recommendation for field release, the leaf beetle *Dirohabda elongata* from China and Kazakhstan, a mealybug *Trabutina mannipara* from Israel, and a foliage-feeding weevil *Coniatus tamarisci* from France.

1. *Diorhabda elongata* (leaf beetle) – control in northern areas.

The *Diorhabda elongata* beetles (Fig. A5-1) have good potential for highly effective, safe and cost-efficient control of saltcear. The subspecies *D.e. deserticola* from Fukang, China and Chilik, Kazakhstan has been extensively tested at Temple since 1992 and also at Albany since 1999. Its ability to develop, reproduce and complete its entire life cycle has been tested on 84 test plant accessions, including 6 species and 22 accessions of *Tamarix*, 4 species of the somewhat related and native *Frankenia*, and 52 species of more distantly related plants, habitat associates, agricultural crops, and ornamental plants (DeLoach et al., 2003a; Lewis et al. 2003a).

These tests, summarized by DeLoach et al. (2003b), demonstrate conclusively that *D. e. deserticola* can feed as larvae or adults, is attracted to and lays eggs on, or completes its entire life cycle only on species of two plant genera - *Tamarix* and *Frankenia*. However, development, attractance to, and oviposition on *Frankenia* in cages was so low that completion of its life cycle on these plants is rare, and they are not expected to sustain a population on this plant in the field. Development and reproduction on the distinctive, exotic, large, evergreen tree, athel (*Tamarix aphylla*), that is a shade tree of some beneficial value in southwestern desert areas, was only 10 to 20% of that on the target saltcedars. The beetle is expected to feed on and colonize athel to a minor extent after release, but not to cause important damage to the trees (Table A5-1).

Table A5-1. Multiple-choice host selection test by larval and adult *D.e. deserticola* from Fukang, China and Chilik, Kazakhstan, 2000, at Temple, TX[a]

	Mean % on each test plant during test, normalized to 100% of total (no. replications)		
Test plant	Larval survival egg to adult	Adults on plants[a]	Eggs laid on plants[a]
T. ramosissima (WY)	29.3 (13)	43.8 (29)	45.7 (35)
T. parviflora (CA)	13.0 (24)	28.7 (4)	33.7 (7)
T. aphylla (TX)	18.0 (15)	27.0 (17)	19.7 (20)
F. jamesii (CO)	6.7 (12)	0.25 (32)	0.93 (35)
F. salina (CA)	12.4 (23)	0.19 (32)	0.00 (35)
F. johnstonii (TX)	4.3 (10)	0.06 (32)	0.00 (35)
F. palmeri (CA)	16.2 (7)	-	-
Total counted: all reps		1,596	8,846

[a]Multiple-choice tests in 3X3X2(h) m outdoor cages (5 tests, 29 reps), small outdoor cages (1 test, 3 reps), (Fukang beetles); or greenhouse in 1.4X1.5X0.5 (h) m cage (Chilik beetles only, only eggs counted, 1 test 3 reps). From Lewis et al., 2003 (Biological Control, May-June 2003).

Diorhabda elongata deserticola has received U.S. Fish and Wildlife Service concurrence, all NEPA clearances, and USDA-APHIS-PPQ permits for release. It was released into field cages from the summer of 1999 and 2000 at 10 sites in Texas, Colorado, Wyoming, Utah, Nevada and California. It successfully overwintered and heavily damaged saltcedar at six of these sites: Pueblo, CO; Lovell, WY; Delta, UT; Lovelock and Schurz, NV; and Bishop, CA. The beetles did not overwinter at the Seymour, TX site, but those added to the cages in the spring heavily damaged the plants during the summer. The beetles were released from the field cages and into the open field in May 2001 at all 6 sites where they overwintered (Pueblo, Lovell, Delta, Lovelock, Schurz, Bishop) and at Seymour. Beetle populations developed in the surrounding saltcedar plants at Pueblo, CO, Lovell, WY, Delta, UT and Lovelock, NV.

At Lovelock, NV these beetles established and reproduced readily in the field. By August 2002 they had completely defoliated all saltcedar over a 2-acre area and numerous adults and larvae were present in an area twice this size. By July 2003, the first generation adults and larvae had defoliated an area of ca. 8 acres. During the spring of 2003, most saltcedar plants had resprouted from the base, and some had resprouted from the upper branches but most of the upper stems had died. During June, adults and larvae had killed most of this regrowth. In the previous field cages, defoliation for 2 years completely killed even larger plants.

At Lovelock, plant kill may exceed 95% within 3 years after release of the beetles. However, at other locations (especially at Lovell, WY) predation by ants has seriously reduced the effectiveness of the beetles. DeLoach and Gould (1997) estimated that 75 to 85% control in natural areas was sufficient to prevent damage to natural ecosystems and to improve water conservation. Populations at Pueblo, CO produced extensive defoliation of ca. 35 nearby plants in 2002 and have extended defoliation to an area 100 m diameter in 2003.

At Lovelock, NV by late August 2003, at the end of the third growing season after release, when large larvae of the 2nd generation had become abundant, the Fukgang, China/ Chilik, Kazakhstan beetles had defoliated 500 acres along 3 miles of the Humboldt River (Fig. A5-2). Similar control occurred at Delta, UT (100 acres defoliated), Pueblo, CO (100 acres defoliated), and at Schurz, NV (30 acres defoliated). Early results indicate that this could be one of the most effective biological control programs ever initiated. Monitoring is essential to determine the effect of this degree of control in improving the native plant and animal communities and to determine if the beetle populations are being restricted by predators or other factors.

2. *Diorhabda elongata* – potential for control in southern areas.

a. Failure of Fukang/Chilik beetles in Texas.

The *D. e. deserticola* beetles did not overwinter in cages at Seymour, Dallas or Temple, TX nor after release into the open field at Seymour, Schurz or Bishop. Beetles placed in field cages in the spring at the Texas locations developed normally and produced another generation of adults by late June. However, this generation did not oviposit, ceased feeding, entered diapause in mid-July, and died during the winter. Observations indicated that the probable cause was that the summer daylength at these most southern sites is too short to prevent diapause. The beetles then starved during the 7 months before saltcedar foliage becomes available in March. These observations were confirmed by our collaborator at Albany, CA, who demonstrated in intensive laboratory studies that *D. e. deserticola* requires a minimum of 14 hr. 45 min. to prevent the initiation of diapause; maximum daylength at Seymour (33.3°N) at the summer solstice is only 14 hr. 21 min., is somewhat less at Dallas, and is 14 hr 10 min at Temple (31.1°N). We conclude that these beetles will not control saltcedar in Texas or in other locations south of ca. 38°N latitude (Lewis et al. 2003 in press; Dan Bean, USDA-ARS, Albany, CA, personal commu.).

b. Host range of southern adapted biotypes of *D. elongata*.

During 2002 and 2003, we received shipments into quarantine of 4 additional biotypes of D. elongata (different from the Fukang/Chilik biotype), from Turpan, China; Crete, Greece; Tunis, Tunisia and Karshi, Uzbekistan. In laboratory tests at Albany, all 4 of the new biotypes appear to be adapted to short daylength south of the 37th parallel. During the fall of 2003, we plan to release the Crete, Turpan and Karshi beetles into field cages at Lake Thomas and/or Beals Creek to determine which overwinters, develops best, and damages saltcedar the most there. Then, the best biotype will be released into the open field during the spring of 2004.

The Crete beetles were collected along the north shore of Crete, at 35°28'N latitude, or similar to that of Amarillo, TX. These appear slightly different morphologically from *D. e. deserticola* and may be a different subspecies. During the summer of 2002, we conducted the full spectrum of host range tests with these Crete beetles as done previously with the Fukang beetles. The host range seemed to be identical to the Fukang beetles previously released except for slightly more development and oviposition on athel and *Frankenia salina*. The second shipment was of *D. e. deserticola* (the same subspecies as the Fukang beetles) but collected near Turpan, China only 100 miles southwest of Fukang. These beetles appear to be identical to the Fukang beetles in every way except for daylength response.

More recent tests, conducted during June and July 2003, Dr. Lindsey Milbrath, ARS, Temple, compared *D. elongata* beetles from Crete, Turpan, Uzbekistan and Tunisia individually but at the same time in paired plant tests (1 saltcedar and 1 *Frankenia* plant together) in small cages outdoors at Temple. These beetles were strongly attracted to saltcedar (Table A5-2), laid several eggs on the cage walls, but placed very few or no eggs on *Frankenia* (Table A5-3).

Table A5-2. Host selection by adult *Diorhabda elongata*: Paired-choice adult tests, Temple, TX, July 2003[a]

	Mean no. adults per plant for each beetle type			
Location of adult beetles	Crete, Greece	Sfax, Tunisia	Karshi, Uzbekistan	Turpan, China
Test 1	*T. ramosissima* X *T. chinensis* vs. *F. jamesii*			
Tamarix ramosissima X *T.chinensis* (CO)	**17a**	**13.2a**	**16a**	**11.2a**
Frankenia jamesii (CO)	**0b**	**0.6c**	**0.6b**	**0.2c**
Cage walls	1b	3.6b	1.6b	3.8bc
Missing/dead	2b	2.6bc	1.8b	4.8b
Test 2	*T. ramosissima* X *T. chinensis* vs. *F. johnstonii*			
Tamarix ramosissima X *T.chinensis* (CO)	**17a**	**14.4a**	**16a**	**9.6a**
Frankenia johnstonii (TX)	**1b**	**0b**	**1.8b**	**0b**
Cage walls	0b	3.4b	0.6b	2.4b
Missing/dead	2b	2.2b	1.6b	8a

[a]Outdoor tests in screen cages 68X53X85 (ht) cm, each cage with 20 beetles (10 males, 10 females) and 2 plants (1 *Tamarix* and 1 *Frankenia*), 3 or 5 replications of each test/beetle type (from research by Dr. Lindsey Milbrath, ARS, Temple, TX).

Table A5-3. Ovipositional host selection by female *Diorhabda elongata*: Paired-choice adult tests, Temple, TX, July 2003[a]

	Mean no. eggs per plant for each beetle type			
Location of eggs	Crete, Greece	Sfax, Tunisia	Karshi, Uzbekistan	Turpan, China
Test 1	*T. ramosissima* vs. *F. jamesii*			
Tamarix ramosissima X *T.chinensis* (CO)	**533a**	**498a**	**545a**	**464a**
Frankenia jamesii (CO)	**0b**	**4b**	**0b**	**0b**
Cage walls	27b	22b	52b	24b
Test 2	*T. ramosissima* vs. *F. johnstonii*			
Tamarix ramosissima X *T.chinensis* (CO)	**446a**	**465a**	**406a**	**355a**
Frankenia johnstonii (TX)	**0b**	**4b**	**5b**	**0b**
Cage walls	36b	34b	71b	62b

[a]Outdoor tests in screen cages 68X53X85 (ht) cm, each cage with 20 beetles (10 males, 10 females) and 2 plants (1 *Tamarix* and 1 *Frankenia*), 3 or 5 replications of each test/beetle type (from research by Dr. Lindsey Milbrath, ARS, Temple, TX).

c. Biology and behavior of southern biotypes. Both the Crete and the Turpan beetles remained active throughout the growing season at Temple and Dallas. At Temple, they oviposited until mid September and were still active in the cages until mid-November. These beetles appear to be insensitive to photoperiod and probably begin diapause in response to cold temperatures in the fall. The Crete beetles overwintered in outdoor cages under nearly natural conditions at Temple, TX with little mortality, emerged during March, and began vigorous egg laying after about a week. The first spring generation of larvae began pupating in early May, about a month earlier than in the northern sites. Only a few Turpan beetles were available and they have not yet overwintered. The Turpan beetles increased to high populations in the field cages at Seymour, TX during July 2003 and now are ready for field release at Lake Thomas.A Letter of Concurrence from FWS was obtained on 13 June and Release Permits from APHIS were obtained on 2 July for release at all requested sites in Texas including Seymour, Merideth Lake, Lake Thomas/Beals Creek, Candelaria, Zapata, and San Jacinto. Turpan beetles placed in a field cage at Seymour, TX in March 2003 increased slowly at first but during July increased rapidly and severely defoliated the saltcedar. These beetles were released into the open field at Seymour on 30 July and placed in cages at Lake Thomas on 31 July. The Crete beetles were placed in field cages at Seymour, Lake Thomas, and at Beals Creek on 8 July. Previous projections (DeLoach and Tracy 1997) suggested that 75 to 85% control of saltcedar was sufficient to salvage most of the water losses and to allow essentially full recovery of native plant and animal communities.

The behavior in the open field of the Turpan and Crete beetles planned for release at Lake Thomas/Beals Creek is still unknown, and is the objective of the present study. However, the Crete beetles overwintered at Temple with very low losses and increased rapidly in cages during the spring of 2003. The Turpan beetles have not yet overwintered in field cages but laboratory tests project that they should. In field cages at Seymour, the Turpan beetles increased to high populations during July, and laid many eggs, a month later than had the Fukang beetles there during 2000, which is an additional generation more than the Fukang beetles, and is a good indication that they can reproduce throughout the summer (the Fukang adults did not lay eggs after June) and can overwinter (the Fukang beetles did not overwinter). These experiments indicate that both the Crete and the Turpan beetles can establish and can control saltcedar in the climatic/daylength zones of Texas, unless suppressed by naturally occurring biotic agents.

3. Clearances and authorizations required

a. Procedures

In order to release exotic biological control agents in the United States to control weeds, several authorizations are required under Federal laws and regulations. Clearances for these actions must be obtained through the Department of Agriculture of each state where releases are desired, and then from the U.S. Department of Agriculture's Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ), through petition to the APHIS-PPQ's Technical Advisory Group for Biological Control Agents of Weeds (TAG). The TAG consists of 13 members from USDA, Department of Interior and other Federal Agencies, and also sends petitions for review to their Canadian and Mexican counterparts. If any endangered species may be affected by the releases, the TAG member from the USDI Fish and Wildlife Service advises APHIS and the petitioner that a Biological Assessment to FWS will be required for FWS review and approval via a Letter of Concurrence (or a Biological Opinion) as authorized under Section 7 of the federal Endangered Species Act (ESA). If approved, APHIS-PPQ then will publish an Environmental Assessment (EA) in the Federal Register for public comment. After review of the public comments, APHIS will publish a Finding of No Significant Impact (FONSI) if their review indicates that the release is justified. After these approvals, APHIS-PPQ issues release permits through each state Department of Agriculture, to the petitioner.

b. Previous Clearances Obtained

All regulatory clearances have been obtained for release of *Diorhabda elongata* into the field in the United States, including Texas, as required under USDA-APHIS-Plant Protection and Quarantine, the National Environmental Policy Act (NEPA), the Endangered Species Act (ESA), and the Texas Department of Agriculture, as follows:

1) Petition to TAG for resolution of conflicts of interest: 19 June 1989 (DeLoach 1989).
2) Reply from TAG, recommending approval: December 1991 (Cofrancesco 1991).
3) Petition to TAG (through state Departments of Agriculture of Texas, New Mexico, Colorado, Wyoming, Utah, Nevada and California for release of *Diorhabda elongata* into the field: 21 March 1994.
4) Approval from TAG 26 June 1995 (Cofrancesco 1995, Lehtonen 1995).
5) Biological Assessment to US-FWS, Region 2, Albuquerque, NM, 17 October 1997 (DeLoach and Tracy 1997).
6) Research Proposal, to US-FWS, Arlington, VA, 28 August 1998 (DeLoach and Gould 1998).
7) Letter of Concurrence, US-FWS, Arlington, VA, 28 December 1998 (Johnson 1998).
8) Environmental Assessment, published in Federal Register by USDA-APHIS, 18 March 1999 (Reed 1999).
9) Withdrawal of Letter of Concurrence, April 1999.
10) Revised Letter of Concurrence, US-FWS, Arlington, VA, 3 June 1999. Approved 10 release sites, one at Seymour, TX and 9 sites in CO, WY, UT, NV, CA, but eliminated all 5 proposed sites in the Pecos and Rio Grande Valleys (Laredo and Big Bend National Park, TX; and Artesia, Holloman AFB, and Bosque del Apache, NM and the site near Phoenix, AZ, and added the site requested at Delta, UT) (Frazer 1999).
11) FONSI, 7 July 1999 (Nave 1999).
12) Release permits from USDA-APHIS 12 July 1999, for Seymour, TX, and 9 sites in other states.

c. Clearances Needed for New Sites

A request to Region 2, U.S. Fish and Wildlife Service, Albuquerque was submitted on 14 February 2003 for 20 new release sites in 6 states, including 6 sites in Texas. The present proposal for releases on the upper Colorado River, at Lake Thomas TX with a subsite near Big Spring, TX was approved by Letter of Concurrence from FWS on 13 June 2003 for release of all *Diorhabda elongata* biotypes at Lake Thomas and Beals Creek. Permits were received from

APHIS-PPQ on 2 July 2003 for release of *Diorhabda elongata* biotypes from Crete and Turpan at all requested sites in Texas.

A second request to Region 2, FWS was sent on 8 April 2004, requesting unlimited releases on the Upper Colorado River above Lake Thomas dam, and above a line on Beals Creek ca. 10 miles east of Big Spring, and to their headwaters. This request also included written confirmation of the telephone approval for a release site at Ft. Stockton, TX.

4. <u>Releases of *Diorhabda* beetles made during 2003 at sites south of the 37th parallel</u>. During 2003, southern adapted biotypes of *Diorhabda* beetles were released into field cages, at 2 sites in California, 1 site in New Mexico, and at 7 sites in Texas. Later in Texas, beetles were released into the open field at the 2 California and 1 New Mexico sites, and at 3 sites in Texas (Table A5-4).

Table A5-4. Field releases of new *Diorhabda elongata* biotypes in southern areas in 2003.

Approved release site	°N latitude	Origin (and latitude) of beetles: date released Crete (35°20′)	Tunisia (34°46′)	Uzbekistan (39°55′)	Turpan, China (42°57′)
		Released in field cages			
Seymour, TX	33°43′	24 Jun			20 Mar
Lake Thomas, TX	32°36′	10 Jul			31 Jul
Big Spring, TX	32°15′	10 Jul		20 Aug	
Candelaria, TX	30°8′				
Ft. Stockton, TX	30°50′				
Kingsville, TX	27°25′	27 Aug	13 Aug		
Zapata, TX	26°58′	5 Nov	26 Aug		
Artesia, NM	33°9′	13 Aug			
Cache Creek, CA	38°56′	22 Aug			
Brantley, NM	32°5′				
San Jacinto SP, TX	29°45′				
Hunter-Liggett, CA	35°57′				
		Release in open field			
Seymour, TX		14 Aug			31 Jul
Lake Thomas, TX		21 Aug			
Big Spring, TX		16 Sept			
Artesia, NM		28 Aug			
Hunter-Liggett, CA		7 Sept			
Cache Creek, CA		29 Oct			

Section A6. Project/Task Description

A. Integration with Umbrella Project on Biological Control of Saltcedar throughout the Western United States

This project is part of the overall program on biological control of saltcedar encompassing at present 9 western states (CA, OR, NV, UT, MT, WY, CO, NM and TX) that began at Temple, TX in 1986. Release of beetles from Fukang, China, Chilik, Kazahstan into field cages began in 2001 in 6 states (CA, NV, UT, WY, CO, and TX) and into the open environment in May 2001 in these 6 states. Releases at 5 sites, 1 each in UT, CO, WY and 2 in NV now are well established. By the end of the 3rd growing season after release (late August 2003) the released beetles had defoliated ca. 500A of saltcedar at Lovelock, NV, 30A at Schurz, NV, 100A at Pueblo, CO and at Delta, UT, and 15A at Lovell, WY; all these sites are north of the 38th parallel.

However, these Fukang/Chilik beetles, that originated in Asia from 43°30' to 44°N latitude, failed to overwinter at the 3 sites south of the 37th parallel and did not establish or damage saltcedar in the spring. This failure was because the summer daylength in these southern areas was too short, causing the beetles to prematurely enter overwintering diapause in early July. Laboratory and preliminary field testing has indicated that different biotypes of *D. elongata* collected from more southern areas in Eurasia (Crete, Greece, 35°20'N; Sfax, Tunisia, 34°46'N; Karshi, Uzbekistan, 39°55'N; and Turpan, China, 42°57'N) are capable of overwintering south of the 37th parallel. During the winter of 2002-2003, the beetles from Crete overwintered successfully and with very little mortality in outdoor cages at Temple, TX (31°6' N).

B. Objectives

The objectives of this proposal are to demonstrate 1) the ability of the 4 southern-adapted biotypes of *D. elongata* (from Crete, Greece; Sfax, Tunisia; Karshi, Uzbekistan; and Turpan, China) to overwinter and establish south of the 37th parallel. 2) The efficacy of these biotypes in controlling saltcedar there, 3) to select the best and fewest number of these beetle biotypes that would give satisfactory control throughout Texas and the Pecos River valley of New Mexico, 4) The herbicidal applications were delayed 2 years from the original plan so saltcedar recovery after spraying may still be insufficient to sustain a beetle release. This will be monitored and if possible in 2007 the beetles will be released but we will not have time in this grant to evaluate the results, and 5) to demonstrate the feasibility of and time required to control saltcedar only with biological methods throughout an entire watershed.

This project is part of a larger project with these southern beetle biotypes underway in southern California, in southern New Mexico, and at 6 other sites in Texas (at Lake Merideth, Ft. Stockton, Candelaria, Zapata, Kingsville, and San Jacinto State Park). The present proposal is the most comprehensive in that it alone includes 5 initial release subsites, unlimited releases over an entire upper-river watershed, integration with other control measures, extensive monitoring of the beetles and their effects on saltcedar, and the effects of biological control of saltcedar on recovery of native plant populations and of wildlife populations.

This is the only project in the U.S. as of this date, that seeks to demonstrate biological control of saltcedar on a watershed scale, through unlimited multiple releases throughout the watershed.

To demonstrate the potential of biological control of saltcedar to improve best management practices (BMP) that relate to increased water quality and quantity, we have selected 5 research/demonstration sites in the Upper Colorado River watershed of northwestern Texas. The Colorado River provides water for cities and towns from its source to the Gulf of Mexico, including Austin. It provides water for several reservoirs and lakes, including Lake J.B. Thomas, Lake Colorado City, Lake E.V. Spence, Lake O.H. Ivie, Lake Buchanan, and Lake Travis. These 5 demonstration sites are all in riparian areas, heavily infested with saltcedar, and all within the jurisdiction of the Upper Colorado River Municipal Water District (CRMWD) headquartered in Big Spring, TX. The CRMWD supplies water to all cities from Abilene to Midland, all of which have been under intense water shortages for the past several years. The shortages are believed to be in part because of the large infestations of exotic saltcedars that use large amounts of water within the Colorado River watershed. The 5 sites are located along the Colorado River upstream from Lake J.B. Thomas dam and along Beals Creek from Moss Lake 10 mi east of downtown Big Spring (Lancaster St.), and including the entire watershed upstream from these two starting points.

The Texas sites range latitudinally from ca. 35°40'(?) at Lake Merideth to 27° at Zapata. The demonstration area covered in this proposal ranges from ca. 32° to 33°52' N (Figure A5-1), and along the Colorado River (upstream from Lake Thomas dam) and all its tributaries (including Lost Draw, Sulphur Draw and Tobacco Draw) and along Beals Creek from its confluence with the Colorado River and including all its tributaries (Buzzard Draw, Mustang Draw, Monument Draw, Seminole Draw and Sulphur Spring Draw) to their headwaters, all in Texas.

The basic experimental design compares the impact of biological control on environmental factors before and after saltcedar control in the same area and also compares the impact of saltcedar control on environmental factors in different areas of saltcedar vs. native vegetation.

The project sites will undergo intensive efforts to achieve 90% or greater permanent control of saltcedar (including that of regrowth and reinfestation), along with natural recovery of native plant and animal communities. The major factors to be measured are the increasing population levels of the biological control agent (the introduced leaf beetle, *Diorhabda elongata*) vs. the decline of saltcedar over time, and the recovery of native plant and animal communities.

Populations of the *Diorhabda* beetles and their reproductive rate, mortality factors and dispersal will be monitored at least each 2 weeks during the first generation after establishment and at least once during the peak of 3rd-instar larvae of each of the expected 3 or 4 subsequent annual generations thereafter. The damage and control of saltcedar, and the impacts on and recovery of native plant communities, will be measured by ground-level observations and measurements annually in the spring and by low-level aerial photography annually in the fall. Species diversity and densities of all birds will be measured three times in saltcedar vs. native vegetation during the spring breeding season, and of all butterflies during June and August.

Data will be entered into an electronic database for storage, retrieval and analysis. A combined Global Positioning System (GPS), Geographic Information System (GIS), and low-level aerial photographic remote sensing will be used to map the project sites and to demonstrate the control of saltcedar obtained over time. A final report will be produced documenting the results of this project. The results also will be presented at scientific, agency and public meetings and will be published in appropriate scientific journals, farm/ranch magazines, and newspapers.

C. <u>Locations to be released</u>

The beetles will be released within "Segment 1" of the Colorado River Saltcedar (CR-SC) Project, which includes saltcedar infested areas of the Colorado River, TX and its tributaries from Lake Thomas dam to their headwaters, as follows:

1. Two subsites at Lake Thomas, one within the shoreline or delta and another ca. 1.2 miles upstream.
2. Three subsites along Beals Creek near Big Spring, 25 miles south of Lake Thomas: two just east of Big Spring and one west of Big Spring.
3. After the first year, unlimited releases along the Colorado and its tributaries from Lake Thomas dam and along Beals Creek 10 miles east of Big Spring and to their headwaters.
4. During Year 5 of the CR-SC project, beetles will be released throughout "Segment 3" to obtain control of regrowth and reinfestation.
5. The area into which the beetles are allowed to disperse without requirement to eradicate them, includes the entire state of Texas, except the Rio Grande valley and that only if Mexico objects to their release there, or if the beetles attack and significantly damage non-target, non-*Tamarix* plants.

The Lake Thomas sites are part of a larger group of one existing site at Seymour, TX and 7 Texas sites and 3 New Mexico sites being requested of Region 2, U.S. Fish and Wildlife Service, Albuquerque, NM, and 8 other sites within Regions 1 and 6. U.S. Fish and Wildlife Concurrence and APHIS approval was obtained in August 2003 for release of any or all *Diorhabda* beetles anywhere in Texas.

9. Ft. Stockton, TX area (Leon Creek, Imperial, and Pecos River near Ft. Stockton).

D. <u>Methods for release</u>

1. <u>Species/biotype to be released</u>.

Along the Colorado River of Texas and its tributaries upstream from Lake Thomas dam (initially along Lake Thomas and Beals Creek) (near Big Spring) we propose to release the biotypes of *Diorhabda elongata* from Crete, into field cages during the fall of 2003. The biotype that overwinters, reproduces, and that controls saltcedar best will then be released into the open environment during the spring of 2004.

Laboratory tests at Albany, CA demonstrated that all these biotypes require substantially less than the ca. 14 to 14 hr 45 min daylength of this area and so should be adapted to overwinter successfully here. In the outdoor cages at Temple, the Crete beetles laid eggs through September and some remained active through November, overwintered in outdoor cages with little mortality during the 2002/2003 winter, and reproduced well the following spring, and overwintered in the open field during the winter of 2003/4 but with unknown mortality. Overwintering ability of the other 2 biotypse has not yet been demonstrated in outdoor cages. Our tests demonstrate that all these biotypes are safe to release, having the same behavior and host range (Tables 2 and 3) as the Fukang/Chilik beetles previously released (Table 1).

2. Source of insects to be released.
Beetles for release may be obtained from 2 sources. First, adults, eggs or larvae from overwintering cages at Seymour, Dallas and Temple, Texas will be released, if sufficient numbers are available. If not enough, these beetles may be allowed to multiply and the following generation may be released. These beetles were obtained from shipments received from overseas, reared in quarantine, and are free of pathogens, parasites or other arthropod species prior to release. Second, beetles may be obtained from laboratory cultures at Albany, CA that also are free of pathogens, parasites or other organisms.

Diorhabda elongata beetles from Crete were released in field cages at Lake Thomas near Site 2 and at Beals Creek Site 2a, and also at Seymour, Dallas and Temple, TX during 2003; populations are being maintained there that can be released at these research sites. Also, the Crete beetles were released in the open field at 3 sites in Texas during 2003, ca. 2,200 at Site 1a (Lake Thomas), ca. 800 at Site 2c (Natural Dam Lake), and 800 at Seymour. Beetles can be obtained from any of these sites for re-release if sufficient populations have developed.

3. Numbers of insects to be released.
At each site, we will release 100 to 200 overwintered adult *D. elongata* in each of 2 cages, with repeated similar releases if needed, to obtain at least 50 to 100 reproducing females in each cage. As soon as authorizations are obtained from FWS and APHIS, releases into the open field will be made at one point at each of Lake Thomas and Beals Creek during the first year, with 500 to 2000 adults and/or large larvae released at each site. During the second and third year, additional secondary releases of 200 to 500 beetles will be made at sites throughout the area upstream from Lake Thomas dam (after final clearance by FWS and APHIS) limited only by the availability of beetles and personnel to distribute them.

4. Cages and surroundings.
The beetles first will be released into field cages at each site. Cages are made of 32-mesh Saran plastic screening, placed over a metal frame, and entered through a zippered door on one side. The cages are 10x10 by 6 to 10 ft high. The bottom of the screen is sandwiched between two 1x6 boards bolted together, buried 4 inches in the soil, backfilled with the soil and tapped firmly in place; this prevents escape from under the cage. The cages are surrounded by a minimum of a 4-strand barbed-wire fence 4 ft high at least4 ft from the cage on all sides to prevent cattle or wildlife from reaching the cages. If other animals are present (such as feral hogs), then "hog panels" or chain-link fence will be used. Some of the initial releases will be inside nylon mesh sleeve bags tied over branch terminals inside the big cages.

The field cages will be located in a stand of saltcedar, or mixed saltcedar/native vegetation, of sufficient extent that beetle dispersal, effect on saltcedar stands, and recovery of native vegetation after control can be monitored. The cages will be located in an area that does not flood, that has limited access by the public, and that is hidden from view by the public. The owner or manager will agree not to apply herbicides or insecticides, or to use mechanical controls or fire to control weeds or brush on the site, or in the nearby area that might adversely affect the beetles in the cages or after release from the cages. The area of saltcedar should extend at least to 1 to 3 miles upstream and downstream, along a lakeshore, or in radius, and should be in an area where saltcedar is dense and extensive enough to be damaging to the native vegetation but where sufficient native seed trees are present to allow rapid revegetation.

5. Schedule and method of releases.

a. General. In year 1 of this grant we will determine the exact location of the release cages, obtain landowner/manager agreements, and cut back the saltcedar shrubs to 2-3 ft high, to promote new shoot growth in the spring. Releases will be made as soon during the spring of the first year as overwintering adults emerge from laboratory cultures or from outdoor nursery sites, which normally occurs from late April to mid-May in nature, and as soon as release permits are obtained. A small to moderate-sized reproducing population will be maintained inside the cages at each new site throughout the 1st year growing season and through the following winter to determine overwintering and as a back-up population in case the released beetles don't immediately establish. These beetles will be transferred to other adjacent cages during the growing season if needed to maintain sufficient good-quality foliage as food for the beetles. Releases can be made as late in the year as the beetles are still active (September or October, based on observations in outdoor cages at Temple) and still allow for the beetles to successfully overwinter.

After the released and overwintered adults have produced larvae and pupae, the 1st generation adults (usually in late June) inside the cages will be released outside the cages and onto healthy plants near the cages. If larvae produced by the overwintering adults are numerous, part of the large 3rd instars also may be released outside the cages. Some of the initial releases into the cages, and of the initial releases outside the cages, will be into sleeve bags placed over the terminals of branches so that oviposition, duration of stages, mortality and rate of increase may be measured. These bags will remain in place until adult oviposition can be confirmed, or if medium-sized 3rd instars are produced; 3rd instars should be allowed to pupate naturally on the soil surface, or in special pupation cages on the soil surface.

After establishment and population increases in the field cages, secondary releases will be made throughout the saltcedar infested areas upstream from Lake Thomas Dam and/or the Beals Creek site near Big Spring. These releases will be of 100 to 500 adults or large larvae placed on open trees in a 10 m diameter area, and not necessarily in cages. The objective is to demonstrate for the first time the effectiveness of biological control of saltcedar on a small riverbasin sized area.

b. Year by year activities. The detailed schedule of year by year activities of releases, monitoring and integration into overall control program are as follows (segments refer to the "CR-SC Project"):

Year 1

Segment 1: Release 50 to 200 beetles into each of two 10x10x6 ft field cages; monitor populations of eggs, 1st, 2nd, and 3rd-instar larvae, and adults weekly. Record predators (ants, spiders, predaceous bugs, and others) and destroy as many as possible. Record other insects feeding on the saltcedar plants or on native shrubs within the cage. If insect populations increase to the point of threatening their food supply, transfer 25 to 50 adults to a new nearby cage. When adults of the 1st generation emerge in mid to late June, place 10 males and 10 females in each of 6 sleeve bags over branches outside the cage; record numbers of eggs laid each week, move beetles to a sleeve cage on a fresh branch and count the eggs again each week until the beetles die. Repeat this process during each generation during the growing season. This is to determine the seasonal cycle and number of generations of the beetles throughout the growing season and the date they enter overwintering diapause (if any). In April, establish point counts for monitoring birds in riparian habitats, 10 points within saltcedar in or near the treatment area and 10 points in a nearby untreated control area of native vegetation.

The vegetation monitoring layout and pre-release vegetation monitoring was done on 28-29 July 2003 at Lake Thomas; 40 marked saltcedar trees were established within a 10 ha sampling area, as specified in the Vegetation Monitoring Plan (Figs. 1 and 2). Annually, monitor plant size, plant condition, and foliage quantity on 4 each 40 cm long branch terminals, percent canopy cover of each grass and forb species in two 1-m square quadrats (one at the trunk and one at the canopy dripline) under each of 40 trees and distance to, size and species of the 5 nearest neighbors of the 40 trees.

Segment 3: Obtain baseline data on vegetation and wildlife monitoring as in Segment 1.

Year 2
Segment 1: In March and April, record data and numbers of overwintering adult beetles emerging within the cages each week. This is to determine the date and size of the overwintering adult population and the health and fecundity of the overwintering females. Measure dispersal of beetles from original release point and damage caused to saltcedar. Repeat vegetation and wildlife monitoring as in Year 1.

Segment 3: No monitoring.

Year 3
Segment 1: Repeat monitoring as in Year 2.
Segment 3: Repeat vegetation and wildlife monitoring as in Year 1.

E. Tasks & Deliverables for this project include the following:

1. Evaluation of Biological Control of Saltcedar (BC/SC) Effectiveness
   - QAPP
   - Monitor BC/SC on 5 project sites
   - Provide additional biological monitoring data: control-insect (*Diorhabda elongata*) seasonal populations in cages and in open field, defoliation and death of saltcedar plants, composition and density of riparian plant community, species diversity and density of sentinel animal species (all birds, all butterflies), weather data

(temperature, rainfall, and lake level), before, during and after BC/SC for the upper Colorado River and Beals Creek of northwestern Texas.

- BC/SC report
- GIS data and maps showing monitoring sites in relation to BC/SC demonstration area.
- Photographs and/or descriptions of before and after project sites

2. BC/SC Education
    - Photographs of BC/SC before, during and after control
    - Copies of articles published in newsletters, newspapers, and in farm/ranch/environmental magazines throughout the project.
    - Presentation and discussion of progress and results at local public meetings, and at agency and scientific meetings.

3. Project Coordination
    - Reports of integration of BC/SC with herbicidal treatments planned in other sectors of the Colorado River Saltcedar Control Project.
    - Reports of coordination of this project with other SC/BC projects in Texas, New Mexico, California, Nevada, Utah, Colorado, Wyoming, Montana and Oregon and with the USDA-APHIS SC/BC implementation program in all saltcedar infested areas north of the 38th parallel.

Subtasks are outlined in Table A6-1 along with a listing of the responsible agency or agencies and an activity schedule.

Table A6-1. Project Plan Milestones.

TASK	PROJECT MILESTONES	AGENCY	START	END
1.1	The ARS, in cooperation with TSSWCB, will develop a QAPP to submit to EPA for approval before data collection is started.	ARS, TSSWCB	June 04	May 04
1.2	The ARS will monitor the effectiveness of BCSC at 5 project sites in northwest Texas. The monitoring will be conducted by ARS personnel and by ARS-trained staff. Monitoring will be conducted at 5 intensively managed sites upstream from Lake Thomas dam and on Beals Creek. The treatment area will receive inoculative releases of biological control agents (the leaf beetle, *Diorhabda elongata*) at all five sites. These sites will be monitored for beetle populations and dispersal, degree of saltcedar control, and native plant and animal species and densities prior to, during, and after control.	ARS	March 04	March 07
1.3	The ARS will maintain a BCSC data base for the project results, including GIS data and photographs.	ARS	November 03	March 07
1.4	The ARS will produce maps of the project sites using GIS.	ARS	November 03	March 07
1.5	The ARS, in cooperation with SWCD's, will prepare and distribute a SCBC Effectiveness Monitoring Report to interested entities.	ARS & SWCD's	March 04	March 07
2.1	The ARS and SWCD will install BCSC demonstration sites for public education.	ARS & SWCD	March 04	March 07
2.2	The ARS and SWCD will include articles regarding this project in annual newsletters to natural resource professionals and riparian area landowners in target watersheds.	ARS & SWCD	April 04	March 07
2.3	The ARS and SWCD will conduct field tours of the project sites throughout the duration of the project.	ARS & SWCD	June 04	March 07
3.1	The ARS will increase riparian landowner awareness of BCSC via media options listed in subtask 2.2 as well as other means that may be made available.	ARS & TAES	February 04	March 07
3.2	The ARS, in cooperation with SWCD's, will enroll the landowners in BCSC, if applicable.	ARS & SWCD	October 03	October 05
4.1	The ARS and SWCD's will work with local media to promote project activities.	ARS & SWCD	February 04	March 07
4.2	The ARS and SWCD's will give BCSC presentations to various groups in west Texas.	ARS & SWCD	February 04	March 07

Section A7: Data Quality Objectives and Criteria for Measurement Data

Objectives of the biological monitoring are that the data will be accurate, representative, comparable with other sites and programs on biological control of saltcedar, and complete. Parameters to be measured are: 1) *Diorhabda* beetle reproductive rate, mortality rate, number of generations per year, overwintering rate and rate of dispersal, 2) damage caused to and any control of saltcedar produced, and any damage that may be caused to native plants, and 3) recovery of native plants and animals after control.

The most critical information is the estimation of *Diorhabda* population levels and dispersal, degree of damage and control the beetles produce on saltcedar, and the effect of biological control on recovery of native plant and bird communities. Information on *Diorhabda* biology and ecology in the field, such as reproductive rate, mortality factors, number of generations per year, and overwintering ability is of secondary importance but is useful in comparing effectiveness in different regions or in increasing effectiveness if less than expected.

Estimated Accuracy of Field Monitoring

To assure accuracy, the counts made by the field technicians will be compared immediately afterwards to counts on the same branch made by the experienced ARS technicians until the field technician counts are within 10% of the counts made by the experienced ARS personnel at the same time and location. Relative percent difference (RPD) will be calculated by the formula:

$$\frac{\text{field technician}}{\text{ARS technician}} \text{X } 100.$$

This procedure will be repeated weekly until the field technician counts are consistently within 10% RPD, and monthly thereafter. Variance of the field monitoring data will be tested by statistical analysis of the data at 80%, 90% and 95% confidence limits.

Accuracy of the field identification of plants and insects by the ARS technicians (Robbins and Tracy) will be determined by comparison with insects from the same collections (location and date) made by taxonomic specialists at SEL, NMSU, TX A&M, or the University of Texas, Austin.

Representativeness

The sites selected are characteristic of western Texas riparian habitats and include mixed native/saltcedar and monotypic saltcedar vegetation community types, reservoir floodplains, and streamsides, upland areas and reservoir shorelines.

Comparability

The sampling conducted here is similar to that at other sites in Texas and to ongoing sampling in 7 other western states, so that comparisons between areas can be made.

Completeness

The project biologists and technicians will collect data on the control insects and their predators and parasitoids, saltcedar control, and native plant and animal communities. Weather conditions may prevent collection of some samples; in each case, documentation/field notes of such adverse conditions will be recorded.

Any changes to the monitoring sites listed in Table A7-2 will be made as an amendment to the QAPP.

Although 100 percent of collected data should be available, accidents, insufficient sample volume, or other problems must be expected. A goal of 90 percent data completeness will be required for data usage. Should less than 90 percent data completeness occur, the Program Manager will initiate corrective action procedure (Quality Control Requirements Section B5).

Data completeness will be calculated as a percent value and evaluated with the following formula:

$$\% \text{ completeness} = \frac{SV}{ST} \times 100$$

where: SV = number of samples with a valid analytical report
ST = total number of samples collected

Database checks for validity will be performed on an on-going basis. Data will be reviewed for abnormalities or any unusual results, prior to entry into the database. Any unusual results will be traced for error sources. In the event no error is found, the data will be assumed normal and appropriate for decision determinations. If an error is found and cannot be resolved, the data will be discarded.

The Project Manager will coordinate with Field Biologists and technicians to ensure that proper protocols are utilized.

Section A8: Special Training Requirements/Certification

All personnel involved in sampling, sample analyses, and statistical analyses have received the appropriate education and training required to adequately perform their duties. No special certifications are required. ARS personnel involved in use of global positioning system (GPS) instruments have been trained in the appropriate use of GPS.

Field personnel will receive hands-on training in insect sampling and habitat assessment by working directly with ARS, TAES and water district personnel prior to sampling/assessment activities. Certifications are not required.

The two ARS technicians assigned to the project (Robbins and Tracy) are highly skilled insect taxonomists already with 3 years experience in identifying the insects on saltcedar and on native vegetation occurring in the sample areas. In addition, one of the technicians (Robbins) is highly skilled in the field identification of the plants and birds that occur in the sampling areas. During the vegetation surveys any part-time or less skilled helpers will work alongside Robbins. Robbins and another equally skilled bird identifier (Johnson, a University Professor of Ornithology) will conduct the bird surveys.

All part-time employees will work closely in the field with the skilled ARS identifiers until they become proficient in identifying the plants and insects required for monitoring.

Section A9: Documentation and Records

The TSSWCB Assurance Officer will be responsible for ensuring that the project staff has the most recent version of the QAPP. When an updated version is produced, it will be emailed as a PDF file to the project staff. The changes will be discussed with the project staff to ensure understanding.

Hard copies of all field data sheets, laboratory data entry sheets, field data entry sheets, corrective action reports (CARs), GPS and GIS data, digital photographs, instrument calibration, and billing receipts will be archived by ARS at the Grassland, Soil and Water Research Laboratory (GSWRL), Temple, TX for at least five years. In addition, ARS will archive electronic forms of all project data for at least five years. A CAR form is presented in Appendix A, a copy of a COC is presented in Appendix A, and copies of GM and field data sheets are presented in Appendix A.

The ARS Project Leader will produce an annual quality assurance/quality control report, which will be kept on file at GSWRL with copies made available upon request. Any items or areas identified as potential problems and any variations or supplements to QAPP procedures noted in the laboratory quality assurance/quality control report will be made known to pertinent project personnel and included in an update or amendment to the QAPP.

Quarterly progress reports will note activities conducted in connection with this project, items or areas identified as potential problems, and any variations or supplements to the QAPP. CARs will be utilized when necessary and will be maintained in an accessible location for reference at ARS. CARs that result in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in an update or amendment to the QAPP.

Section B1: Sampling Process Design (Experimental Design)

Sampling sites for measuring control insect populations and effects of control of native plant and wildlife communities are selected, and baseline data taken, before the beetles are released, and periodically thereafter at the same sites. Natural dispersal of the beetles beyond the original sampling areas is determined by ground surveys along the river valley made at the peak of each adult generation, and annually by remote sensing.

A. Sampling Area. The sampling area includes all saltcedar infested riparian areas along the upper Colorado River and along all of Beals Creek in northwestern Texas, lying west of 100° W to near the New Mexico line and between 32° and 34° N latitude (Fig. B1-1, Fig. B1-2). This biological control is the uppermost part (Segment 6) of the Colorado River Saltcedar Control Program (Table B1-1).

Table B1-1: Sampling Sites Locations Monitored by ARS

Figure #, Site	Latitude & Longitude	Subwatershed & General Location
Fig. B1-1	32°-34°N, ____-_____W	Colorado River, Northwest TX (county – city maps)
Fig. B1-2	32°-34°N, ____-_____W	Water District, Hydrologic Accounting Units
Site 1a, Fig. B1-3 Site 1b, Fig. B1-3	32.6048°N,-101.216°W 32.6098°N,-101.238°W	Lake Thomas (low resolution 1996 image)
Site 1a, Fig. B1-4		Lake Thomas floodplain (low resolution 1996 image) (10 ha circle/40 tree sample plot)
Site 1a, Fig. B1-5		Lake Thomas lakebed (low-level aerial photograph, 2002)
Site 2a, Fig. B1-6 Site 2b, Fig. B1-6	32.2509°N, -101.386°W 32.2592°N, - 101.427°W	Beals Creek, Higgins Ranch (Site 2A) Beals Creek (low resolution 1996 image)
Site 2a, Fig. B1-7		Beals Creek (low-level aerial photograph, 2002)
Site 2c, Fig. B1-8	32.2667°N, - 101.661°W	Beals Creek, New Dam Lake/Buzzard Draw (low resolution 1996 image)

Two main intensive sampling areas are included, the Lake Thomas area (Colorado River) and along Beals Creek from ca. 10 miles east to ca. 15 miles west of Big Spring). Additional release sites for *Diorhabda elongata* beetles upstream from these areas will be added as concurrence is obtained from the U.S. Fish and Wildlife Service, as Release Permits are obtained from USDA-APHIS-PPQ, and as beetles become available for additional releases. The monitoring/sampling area will be expanded as the beetles multiply and spread and as new sites are established.

The *Diorhabda* beetles released during 2003-2004 at Sites 1a and 1b (Lake Thomas), 2b (Big Spring, Beals Creek, Sewage Treatment Plant) and 2c (Big Spring, Beals Creek, Buzzards Draw) failed to establish but additional attempts there and at other locations are planned for 2007. Site 2a (Big Spring, Beals Creek, Higgins Ranch) has established strongly, and the beetles increased greatly in population and area of saltcedar defoliated during 2004, 2005 and 2006, with 25 acres of saltcedar stands defoliated by October 2006, with beetle populations reaching several million.

The other sites have been temporarily abandoned and beetles from Site 2a (Higgins Ranch) will be used to re-establish them and to establish additional sites during 2007 and thereafter. We anticipate that landowners will be invited to collect and redistribute beetles on their own lands during 2007, under our supervision.

Site 1 (Lake Thomas area). Site 1 includes the area from Lake Thomas dam upstream for a distance of ca. 6 miles (Fig. B1-3). Two subsites are located here, Site 1a in the floodplain of Lake Thomas and Site 1b in a non-flooding area 1.2 miles upstream from Site 1a.

Site 1a (Lake Thomas floodplain). Site 1a lies in the upper floodplain, above the present lakeshore and ca. 0.3 miles below Murphy's School Road, and between the north and south bluffs that demarcate the floodplain (Fig. B1-3). This site also will measure the ability of the beetles to recover and continue to control saltcedar in case the lake level rises and the site floods.

The area of initial intensive sampling is a circular 10 ha area, with a center point just south of a small intermittent stream, ca. 0.5 km east of Murphy's School road. This consists of an inner circular area of 1 ha, radius 56.4 m; a middle concentric circular area of 2 ha, lying between 56.4 m and 97.7 m from the center point, and an outer 7 ha circular area lying between 97.7 and 178.4 m from the center point (Fig. B1-4, B1-5). Experience at Lovelock, NV and Pueblo, CO indicates that the beetles probably will remain within this area for 2 years after release. This area contains 40 sentinel trees that will be monitored for beetle population and for the damage they produce on the plants.

A second method of sampling/monitoring will consist of sampling the vegetation and beetle populations along transects. Four permanent transects will be established, 2 parallel to the river valley (approximately east/west) and 2 perpendicular to the valley (approximately north/south), all originating at the center point of the 10 ha circle. The perpendicular transects will extend to the bluffs that demarcate the floodplain and the parallel transects will reach to the lakeshore at the dam to the east and to ca. 1 mile upstream to the west. These E/W transects will be extended as needed to record the advancing front of beetle dispersal. One or two additional east/west transects will be added as the beetle population disperses. During the 3rd year after release, the dispersal of the beetles is expected to require these E/W transects to extend 2-3 miles from the center release point.

Site 1b (Upland, upstream from Lake Thomas). Site 1b will be located ca. 1.2 miles upstream from the center point of Site 1a, in an area that does not flood (Fig. B1-3). This is a fall-back site in case Lake Thomas fills and Site 1a floods. It also is one of the 2 primary sites for the beetle dispersal study by the Texas A&M Master's student, Jeremy Hudgeons. The sampling layout will be the same as Site 1a, with the concentric circles enclosing a 10 ha area and with 4 similar transects. The transects will be established during the spring of 2004 before the beetles are released. The 10 ha area with 40 sentinel trees will be established only if Site 1a is lost (this method is more time consuming and less efficient for measuring beetle dispersal and spread).

Site 1 Extended Sampling Area. After the 2nd year after release of beetles, and if beetle dispersal warrants it, spot sampling will be conducted at appropriate distances upstream and downstream as needed to document beetles dispersal, damage to the plants, and amount of saltcedar defoliated or killed. The spatial frequency of spot samples will depend on the patches of saltcedar and accessibility of sites, and may vary from each 100 m to each 500 m to each 1 km. Since only adult beetles disperse for distances greater than ca. 50 m, this sample is needed only at the peak of each adult generation, ca. April 15 (overwintering adults), ca. May 30 (1^{st} generation adults), June 30 (2^{nd} generation adults), August 30 (3^{rd} generation adults), and October 15 (4^{th} generation adults).

Site 2 (Beals Creek/Big Spring area). Site 2 includes areas along Beals Creek (a tributary of the Colorado River) from ca. 10 miles east of Big Spring to ca. 20 miles west of Big Spring. Three subsites are located here.

Site 2a. This site is located on the Higgins Ranch, ca. 6 miles east of Big Spring, along the northern side of the Beals Creek floodplain (Figs. B1-6, B1-7). The sampling procedure will be similar to that at Site 1a but with only the transects being established initially, at or before the beetles are released. The saltcedar here occurs in small patches, scattered trees, and as a narrow strip along both sides of the creek.

Site 2b (Sewage Treatment plant). This site is located at the Big Spring Sewage Treatment plant, ca. 3 miles east of Big Spring (Fig. B1-6). This is a ca. 55 ha area of dense, almost monotypic saltcedar, 4 to 6 m tall. Both a 10 ha circle of 3 concentric rings and 40 sentinel trees, and the 4 transects will be established here, as in Site 1a. If the stand here is too dense for feasible, practical establishment of the 10 ha circle, then only the transects will be established.

Site 2c (Natural Dam Lake). This site is located on private property along Buzzard Draw at Natural Dam Lake on Beals Creek, ca. 9 miles west of Big Spring (Fig. B1-8). Transects for sample beetle populations and damage to saltcedar will be established here and the 10 ha concentric circle also will be established if its establishment at the Sewage Treatment Plant is not feasible.

Site 2 Extended Sampling Area. As beetle populations reproduce and disperse, extended spot sampling will be conducted as described for Site 1. This sampling area will extend from Mass Lake, ca. 10 miles east of Big Spring to 10 miles up Mustang Draw and Sulphur Spring Draw, west of Natural Dam Lake.

B. Sampling criteria.

1. Probability based or hot spot. The sampling encompasses 1) a comparison of saltcedar vs. native vegetational areas, 2) comparison between areas where control agents are present vs. uninfested control areas, and 3) before and after control in the same area. The probability based sampling includes both the 10-ha area of 40 sentinel trees and the transect lines for measuring vegetation, beetle populations, and control achieved. Wildlife population sampling also is probability based, consisting of 10 point counts (replications), at 3 different times, and at different locations (Lake Thomas, Sewage Treatment Plant, and Seymour).

2. Average or hot spot. The sampling plan is mostly probability based but also includes methods for finding hot spots where the beetles have dispersed naturally and founded new colonies.

3. Reference background populations. Reference background populations are included in saltcedar uninfested areas for wildlife sampling and for vegetation sampling. Control insect sampling (*Diorhabda* beetles) and degree of control obtained is based on pre-release baseline data, data during control, and data after control in the same area.

4. Sampling frequency. Most sampling is done periodically, from twice weekly to once each generation for the control beetles and annually for wildlife and native plant communities.

5. Periodical or continuous sampling. Most sampling is done periodically visiting established sites. As the beetles disperse, surveys will extend outward from the original sites to document the rate of beetle dispersal and of saltcedar control.

6. Homogenous or heterogeneous target populations. The area-wide native plant, saltcedar, and wildlife populations are heterogeneous, but occur in small to large patches or in mixed stands. Wildlife sampling is divided into homogenous areas of native vegetation, monotypic saltcedar, and mixed native and saltcedar. Insect and plant sampling is stratified by sampling on saltcedar trees within the mixed stand and separate observations of damage to non-target plants.

7. Monitoring of insect and plant populations. The major cost of this Proposal is for monitoring the increase, dispersal, mortality factors, seasonal abundance and effectiveness of the beetles in controlling saltcedar; and the effect of control in recovery of native vegetation and wildlife populations. This monitoring is required by our Letter of Concurrence from FWS and our release permits from APHIS. The few (300 to 500) beetles to be released are expected to increase to hundreds of thousands, to disperse throughout the floodplain of Lake Thomas, and to control the saltcedar over the original sample area within the 3 years of this study, and to be poised to control saltcedar in the remainder of the Texas Colorado River watershed. This can occur at extremely little cost of only a few thousand dollars needed to redistribute the beetles from sites of abundance, from which the beetles spread naturally into nearby areas. The release site for the Fukang beeltes near Lovelock, NV now seems poised for just such a degree of control throughout the saltcedar infested Humboldt River and Basin, and in only 3 years since their initial release in May 2001. On the other hand, certain biotic factors such as predation (especially by ants) and parasitism, or lack of adaptability to the climate/ecology of this region, may seriously reduce their effectiveness. The monitoring program is essential to determine which direction control will go and to determine the cause and how to correct it if control is not successful.

Monitoring of the beetles and their reproduction, mortality and effect on saltcedar, of vegetation structure and composition, and of bird and butterfly species diversity and populations in both native and monotypic saltcedar, and mixed native/saltcedar communities, was conducted at Seymour during the 2002 growing season, and methodologies are now well developed. Remote sensing of the Seymour site has been done now for 2 years.

Table B1-2. Biological, Ecological and Control Monitoring Breakdown.

Control insect populations and area of dispersal
5 sites (Lake Thomas, Big Spring)
2 locations

Control insect biology

In sleeve bags	reproductive rate/seasonal abundance/generations: no. eggs per female, no eggs per mass, no. 1st, 2nd, 3rd instar larvae, adults
In cages	overwintering

Damage to saltcedar and other plants
2 sites – Lake Thomas, Buzzard Draw
3 methods – 10 ha circle, transects, remote sensing
4 times/yr. (each generation) X 2 yrs

Recovery of native plant communities
2 sites X 2 yrs = 4

Recovery of native animal communities
Birds – 2 sites X 3 dates X 2 yrs = 12

Section B2: Sampling Methods

The sampling process includes 6 methods for collecting data. Information on the seasonal development, mortality, rate of increase and initial dispersion of the beetles and of their effect on saltcedar plants is obtained by 2 methods: 1) In field cages containing the beetles (paragraph A.1, below) and 2) in the open field from a 10 ha circle of 40 sentinel trees (paragraph A.2, below). Two different methods are most useful after the beetles have dispersed, 3) transects from the beetle release point (paragraph A.3 and A.4, below), and 4) remote sensing (paragraph C.2, below). Two methods are used to measure ecosystem effects after control: 5) recovery of native vegetation (paragraph E) and 6) effects on wildlife (paragraph F, below). Water savings resulting from saltcedar control is determined from records of the Upper Colorado Municipal Water District.

A. Measurements of field biology and ecology parameters and populations.

The objectives are to compare damage to saltcedar by the *Diorhabda* beetles with baseline data before release of the beetles, and to compare species density and diversity of native plants, birds and butterflies between saltcedar and native plant communities before release of the beetles, and after control of saltcedar. Most of this is *in situ* counting of the different insects and life stages, or of birds and butterflies, or the counting and measurement of plants, all of which occur under natural conditions in nature. Parameters to be measured are reproductive rate, mortality factors and rate of increase, dispersal, overwintering, predation and parasitism.

The rapid expansion of the beetles at the Higgins Ranch (Site 2a) made the use of the concentric-ring sampling method impractical. We now are using the monitoring system of transects radiating outward from the release point at Site 2a, and beginning in 2007 along Beals Creek upstream and downstream as far as the beetles move. We will also use the Extended Sampling Area from ground surveys and aerial photography. The method of sampling will be "2-minute counts" of adult and larval beetles together with visual estimate of percent defoliation in rapidly expanding areas. In areas of more intense increase, the sampling unit will be 4 x 4 m quadrats, counting adults, eggs and larvae; predators, and estimated percent damage of each quadrat, as is now being done at Site 2a based on counts of nine 1-m long branches per quadrat.

Because of the failure of several sites to establish, we are placing increased emphasis on demonstrating best methods of release to obtain establishment. The methods being evaluated are site selection, time of year to release, numbers to release, and type of cages to use. We plan to refine these methods during the remainder of the grant period. Preliminary results indicate that we should release early in the year (May-June), release large numbers (500-1000 or more), release in our large (10 x 10 x 6-8 ft high) cages or in a group of ca. 20 sleeves with 25 adults per bag, and release by simply removing the cage or bags without disturbing the beetles.

1. Field cages. The beetles first will be released into field cages at each site. Cages are made of 32-mesh Saran plastic screening, placed over a metal frame, and entered through a zippered door on one side. The cages are 10x10 by 6 to 10 ft high. The bottom of the screen is sandwiched between two 1x6 boards bolted together, buried 4 inches in the soil, backfilled with the soil and

tapped firmly in place; this prevents escape of beetles or entry of predators underneath the cage. The cages are surrounded by a minimum of a 4-strand barbed-wire fence 4 ft high at least 5 ft from the cage on all sides to prevent cattle or wildlife from reaching the cages. If other animals are present (such as feral hogs), then "hog panels" or chain-link fence will be used. Some of the initial releases will be inside nylon mesh sleeve bags tied over branch terminals inside the big cages.

The field cages will be located in a stand of saltcedar, or mixed saltcedar/native vegetation, of sufficient extent that beetle dispersal, effect on saltcedar stands, and recovery of native vegetation after control can be monitored. The cages will be located in an area that does not flood, that has limited access by the public, and that is hidden from view by the public. The owner or manager will agree not to apply herbicides or insecticides, or to use mechanical controls or fire to control weeds or brush on the site, or in the nearby area that might adversely affect the beetles in the cages or after release from the cages. The area of saltcedar should extend at least to 1 to 3 miles upstream and downstream, along a lakeshore, or in radius, and should be in an area where saltcedar is dense and extensive enough to be damaging to the native vegetation but where sufficient native seed trees are present to allow rapid revegetation.

The date of occurrence for each life stage (egg, larvae, pupae, adult) and number of eggs laid will be sampled periodically both inside and outside the cages once or twice weekly, with samples that estimate reproductive parameters and populations. Measurements of reproduction may be made by placing newly emerged pairs (male/female) of beetles in sleeve cages over healthy branch terminals and observing them twice weekly.

Overwintering is determined from observations of spring emergence in the 10 X 10 ft field cages or, during the 2nd or 3rd year after release after large populations have developed, in the open field.

2. <u>10 ha circle of sentinel trees, open field</u>. To measure beetle populations and their effects on saltcedar by this method, 40 trees are randomly selected in a nested design within a 10 ha stand of saltcedar: 2 or 3 trees in each quadrant of a 1 ha area (56.4 m radius); 2 or 3 trees in each quadrant of an additional concentric area of 2 ha (56.4 to 97.7 m from the center), and 5 trees in each quadrant of an additional concentric 7 ha (97.7 m to 178.4 m from the center).

The vegetation monitoring layout and pre-release monitoring was done on 28-29 July 2003 at Lake Thomas; 40 marked saltcedar trees were established within a 10 ha sampling area, as specified in the Vegetation Monitoring Plan (Fig. B1-2). Monitor plant size, plant condition, and foliage quantity on 4 each 40 cm long branch terminals, percent canopy cover of each grass and forb species in two 1-m square quadrats (one at the trunk and one at the canopy dripline) under each of 40 trees and distance to, size and species of the 5 nearest neighbors of the 40 trees. Each 2 weeks from overwintering emergence of adults until June 30, count and record numbers of adults, each instar larvae and eggs of *D. elongata* on each of four 40-cm long branches (one each in N, E, S, W direction) at head height or higher, on each of the 40 labeled and numbered sentinel trees.

Foliage condition and damage also will be recorded for each sampled branch and for the whole tree (foliage color, % damaged or destroyed). Photographs will be made of each branch and of each whole tree during May (before much beetle damage) and during late August or September (maximum beetle damage). Photos will include the tree number, branch number, and date.

3. Along transects, open field. Also, *Diorhabda* occurrence and population estimates will be made periodically during the year along the transects. A visual examination will be made of each saltcedar tree along the transect for presence or absence of beetles. Absence will be noted but no measurements of branches made. If beetles are present, they will be counted on four 40-cm branches per tree (adults, larvae and eggs) and this recorded and used to estimate the total number of beetles per tree, also recorded. Condition and damage to the tree will be recorded. If beetle populations appear to be rather uniform along certain areas of the transect, then only sample trees need be sampled, such as 1 tree each 10 or 20 m or each 5th or 10th tree. This scope of sampling is expected to be sufficient for at least 2 years. As the beetles disperse up and down the river after the 2nd and 3rd year after release, the transects will be lengthened, or spot examinations made ahead of the advancing beetle front to define the rate of dispersal and damage to saltcedar.

When pheromone traps, presently under development, are available, they will be used to determine beetle presence far in advance of the moving front of damage.

4. Extended area. As beetle populations disperse beyond the 10 ha circle sampling area, surveys will be made upstream and downstream in saltcedar infested areas along the Colorado River, Beals Creek, and their tributaries and draws. Surveys will be made by walking along transects through beside saltcedar stands. When *Diorhabda* beetle populations are found, beetle populations and damage to saltcedar will be counted and recorded as in paragraph A.3 above, on from 3 to 10 trees and located by GPS on aerial remote sensing photographs.

5. *Diorhabda* parasitism. Possible parasitism of the *Diorhabda* beetles will be measured by rearing field-collected eggs, medium and large larvae, and adults in the laboratory and collecting the parasites that emerge. Samples will be collected during May, July and September at Site 1A (Lake Thomas) and 1C (Natural Dam Lake). At each site on each date, from 10 to 50 egg masses, 20 to 100 medium-sized larvae, and 20 to 100 adults will be collected from the field, held in small, clear-plastic boxes with fresh saltcedar foliage for food, returned to the ARS Temple laboratory within the next 1-2 days. The eggs, larvae and adults will be held separately for each site and each date, but will be combined for the different trees or sub-collections at a site. Collections will be labeled by site and date of collection. At the ARS Temple laboratory, the eggs, larvae and adults will be reared on fresh saltcedar foliage of potted plants until they die, or the eggs hatch, or the larvae pupate. The dead individuals will be examined under the microscope for evidence of parasitism and any parasitoids that emerged in the holding cages will be collected. Percent parasitism of each parasitoid species will be calculated and parasite species will be identified by specialists at SEL, TAMU or NMSU. Identified voucher specimens will be maintained at the ARS Temple laboratory.

6. Predation. Predation is measured by observation of predators on sampled terminals. The major predators are expected to be spiders, ants, predaceous bugs (hemipterans), lady beetles and lace wings. Birds will be observed for predation on larvae and adults. Differences in survival of eggs and larvae in the cages vs. survival in the field may be used to estimate overall predation in the field, although losses from the larvae crawling away or falling often make such comparisons difficult.

B. Sampling Other Insects and Arthropods.

Observations will be made of the presence, population size, and of the type and amount of damage caused by insects other than the *Diorhabda* beetles that attack saltcedar. The major other damaging insects are a leafhopper (*Opsius stactogalus*) and a scale insect (*Chianapsis etrusca*) that are host specific natural enemies of *Tamarix* in the Old World that were accidentally introduced into the U.S. along with *Tamarix* many years ago. Also, several native North American foliage-feeding and stem-boring insects occasionally damage saltcedar. The damage by all those insects can compound the effects of damage by the *Diorhabda* beetles and can mask the effect of the *Diorhabda* beetles.

Twice during each growing season, in May-June and August-September, samples of all non-*Diorhabda* insects and other arthropods will be taken in the field and returned to the laboratory for identification to species under a dissecting microscope and for measuring the abundance of each species. These samples will be taken at the two main sampling areas: at Site 1A at Lake Thomas and Site 3C at Natural Dam Lake. Samples will be taken from 10 saltcedar trees, 5 willow trees and 5 baccharis shrubs in each area. Samples will be taken by vigorously shaking one 40 cm-terminal branch from each plant for 10 second into our standard 5-gal plastic "shake bucket".

C. Assessment of damage by *Diorhabda* beetles to saltcedar.
(See changes in Section B2, paragragh A, paragraph 1 and 2 above).

1. Ground-level monitoring. Damage to saltcedar in the field will be estimated within 3 weeks after the peak population of large, 3rd-instar larvae (which cause the majority of the total generational damage), at approximately June 15 and September 15.

a) In 10 ha circle. Visual estimates of the categories of damage to each tree will be made on the 40 sentinal trees within the 10-ha-circle sampling area after release of the beetles, and along the transects in years 3 and later (as described in paragraph A.2, above) and annually during mid- to late spring throughout this grant period. Damage caused to the saltcedar plants will be made during sampling of beetle populations and dispersal. Visual observations will estimate percent defoliation or foliage browning caused by beetle feeding, and length of dieback of branches and percent of branches affected on sampled trees.

b) Along transects. Samples of vegetation and *Diorhabda* beetles will be taken along 4 transects (8 if time permits), within the 10 ha circle. The transects will originate at the center release point and extend outward 178.4 m, two transects will extend parallel to the river (roughly east and west), one upstream and one downstream. The other two will extend perpendicular to the river one approximately south and one approximately north of the river. As the beetle infested area expands, the east transect will be expanded to the dam and the west transect to Murphy School road, and the north and south transects to the bluffs defining the floodplain.

Along the transects, the distance from the center point, and area of the transect line covered by each species of shrub or tree will be recorded, along with composite grass/forb identification and density for each 10 m segment. This will be done annually, in May or June.

c) In extended areas. As beetle populations disperse beyond the 10 ha circle sampling area, surveys will be made upstream and downstream in saltcedar infested areas along the Colorado River, Beals Creek, and their tributaries and draws. Surveys will be made by walking along transects through saltcedar stands. When damage from *Diorhabda* beetles is found, condition and damage on four 40-cm branches, and of the whole tree, will be made on 3 to 10 trees as in paragraph A.3 and paragraph C.1.b, above. These areas will be included in the annual remote sensing survey as in paragraph C.2, below.

2. Remote Sensing. Remote sensing by low-level, 9" aerial or video photography, will be done annually to provide a highly accurate measurement of the area of saltcedar defoliated by the *D. elongata* beetles. This technology already is well developed by our cooperator at the Kika de la Garza Subtropical Agricultural Research Center, Weslaco, TX.

Remote sensing technology that can distinguish healthy saltcedar from other vegetation is much more difficult than that for distinguishing defoliated, brown saltcedar from other growing, healthy vegetation. If or when this technology is developed, it will be used to map existing saltcedar areas, also funded from sources other than this grant.

D. Damage by *Diorhabda* Beetles to Non-target Plants.

Effects on nearby non-saltcedar plants will be carefully observed during the dispersal measurements. Observations include any adults, eggs or larvae present, observations of feeding, population of beetles present, and amount of damage. All plants will be observed, but especially *Frankenia* and athel (*Tamarix aphylla*) if any grow in the release area.

Any attack by the *Diorhabda* beetles on native vegetation in the field will be recorded from examination of at least 10 plants of native shrubs or trees growing within areas of beetle attack on saltcedar plants near the sample are. Any attack on forbs and grasses will be recorded during the annual monitoring of these species in $1 m^2$ quadrants under the saltcedar trees.

E. Effect of Biological Control on Recovery of Native Plant Communities.

Species diversity, size and canopy cover of native plants are monitored before, during and after biological control of saltcedar. This monitoring is done within the 10 ha circle, along the transects, and by remote sensing.

Sampling of native plant communities before and after control of saltcedar. The increase in size and density of native and other non-saltcedar plants will be measured during and after biological control, compared with baseline data obtained before the beetles were released. Measurements of the size and density of these plants will be obtained from: the ongoing sampling within the 10 ha sampling circle and along the transects, especially of the "nearest neighbors" to saltcedar; the ongoing sampling of forbs and gasses from the 1 m^2 quadrats taken underneath the canopy of saltcedar and at the canopy dripline, and the annual remote sensing survey, combined with ground truthing.

These measurements will be made annually during the period of this grant and periodically for several years thereafter.

Parameters to be measured for each saltcedar tree include:

1) Height,
2) Canopy diameter in two directions,
3) Nearest 3 neighboring trees greater than 1 m high: identification, distance from the sentinel tree, height and canopy diameter of each nearest neighbor.
4) Foliage density, measured by a light bar averaged for 10 to 20 points under each tree, and reference readings of open sky beside each tree, made between 10:00 am and 3:00 pm.
5) Canopy cover of understory vegetation, measured for two 1-m square quadrats for each tree, one near the trunk near the densest area of foliage and one just beyond the canopy drip line. These are permanently marked and the same quadrats are sampled each year. Visual estimates of the percent canopy cover of plant each species growing within the quadrat is recorded.

Measurements along transects are the same as within the 10 ha circle (paragraph A.2, above) except that quadrats of forbs and grasses are not measured.

Comparisons will be made annually in mid- to late spring of the amount of saltcedar control (% defoliation, canopy cover, number of dead or resprouted saltcedar trees), and of the size of native vegetation, in relation to pre-release baseline surveys.

F. Effect of Biological Control on Recovery of Native Animal Communities.

1. Comparison of saltcedar vs. native vegetation. Sampling of wildlife populations is conducted annually within the release area, beginning the first year to establish base-line data and continuing annually throughout the project. Monitoring will include birds and butterflies. The surveys are conducted to measure change in population over time as saltcedar is controlled and, if possible, between an area of near monotypic saltcedar and an area of native vegetation free of saltcedar. Basic monitoring of birds, butterflies, and vegetation is done by identifying the individuals by sight and counting them in place. Samples usually are not taken.

a. Birds. Monitoring of bird species diversity and density will consist of 10 point counts in each of 2 or 3 vegetation types: 1) monotypic saltcedar, 2) native, free of saltcedar, and 3) mixed saltcedar and native, done three times during the breeding season each year. At each point, all birds seen or heard during 5 minutes will be recorded. Each point count is 100 m diameter, separated from other point counts by a minimum of 100 m. One skilled bird observer (with a second person for safety) usually can survey 10 point counts in one day, working from dawn to ca. 9:00 or 10:00 am.

b. Butterfly communities. Butterfly species identity and abundance will be sampled along transects through an area. Each area count is a 100 m transect, along which the surveyor moves slowly, separated from other transects by 50 m or more. Butterflies will be sampled during late morning or afternoon.

2. Comparison of saltcedar infested areas before and after control. Comparisons of bird and butterfly species diversity and density will be made annually in saltcedar infested/controlled areas and in native vegetation areas in relation to diversity/density baseline data before saltcedar control began.

G. Weather Monitoring.

Temperature and rainfall will be monitored continuously *in situ* by "Hobo" recorders at the sites. These data are recorded each minute and will be downloaded onto a notebook computer monthly or more often, during site monitoring by the ARS technicians coming from Temple. The recorders will be located in a secure area, protected by fences from livestock and wildlife and on private property protected from public access, and in areas not likely to flood. More complete weather stations will be located at the 2 sites where *Diorhabda* dispersal data is recorded. These weather stations will record wind direction and velocity and solar radiation in addition to temperature and rainfall.

H. Composited samples.

Some samples will be composited, such as identification and canopy cover of grasses and forbs in the quadrats and along transect segments. Other samples are handled individually.

The Project QA Manager will assure that personnel are proficient at identifying and counting the plants, insects and birds to be monitored.

The insect collecting equipment (insect aspirators, shake buckets, and plastic boxes or small cages) used to transport insects to Temple for further study, will be cleaned with soap and water and air dried after each use. No special disposal of byproducts is necessary.

If problems occur in the field sampling process, responsibility for corrective actions will be in the following order: ARS Project Manager/QA Manager (DeLoach), ARS Project Biologist (Milbrath), TAMU Cooperator (Knutson), and ARS Lead Technician (Tracy).

If a site is destroyed (as by accidental herbicide or insecticide applications or by fire) the sampling being conducted then will be moved to one of the other sites at Lake Thomas site B or Big Spring sites A, B, or C, or in the case of destruction of the bird monitoring sites to the ongoing monitoring site near Seymour, TX (not a part of the present study). If a site is temporarily destroyed by flooding, sampling will resume as the flood waters recede, as this is a part of the natural environmental system. If the collected data sheets are lost or destroyed, replacement monitoring will be conducted as soon as possible after the loss.

I. Sampling Equipment and Instrumentation Needs.

- Temperature recorders
- Rain gauges
- Wind direction and speed recorder
- Laptop computer
- Light bar
- Tape measure, 100 m
- Compass
- Insect sweep nets
- Outdoor cages, 10x10 ft X 6 ft high or larger, 32 mesh Saran plastic screening
- Nylon sleeve bags
- Step ladder, 10 ft aluminum
- Plant press
- Insect killing jars, specimen envelopes, vials and alcohol
- Dissecting microscope
- Hand clippers and loppers
- Clipboards, rain protected

Section B3: Sample Handling and Custody Requirements

Data collected from all field monitoring and sampling, and from all laboratory tests, is recorded on field or laboratory data forms as it is being collected. All data sheets are labeled with type of data, date, location, site number, and person(s) collecting the data. Data sheets will be held on field clipboards during collection, transferred to labeled data-sheet binders immediately after each collection trip, and stored at the ARS Temple laboratory for entering into computer data bases and analyzed at the end of the growing season.

Samples collected in the field for identification or further study will be labeled with tags bearing the date, location and sample number. Insect or plant specimens sent to other locations for identifications are assigned a specimen number that is attached to the specimen and recorded in a log book maintained at the ARS Temple laboratory (see attached forms).

Voucher specimens of the various insects and plants sampled, with their date and location of collection will be maintained by ARS at GSWRL, Temple, Texas. These will include:

a) The control insect (*Diorhabda* leaf beetles)
b) Insect parasitoids or predators of *Diorhabda*
c) Plant specimens of saltcedar and native plants

The soft-bodied insects and spiders, and insect larvae will be transferred to vials of 70% ethanol, and hard bodied insects, moths and butterflies will be killed in a killing jar and transferred to glassine envelopes, and beetles and other hard-bodied insects will be killed and placed in dry vials for return to the laboratory. Hard-bodied insects will be preserved on insect pins and held in an insect storage cabinet. A few identified voucher specimens will be labeled with data and location collected, host plant, insect name and identifier and retained at the Temple laboratory for future comparison with other field collected insects. Each sample will be identified by sample number, site name, date of collection, plant species, tree number (tagged and GIS located), and collector's name. Field record sheet also will record this information and weather conditions and time of day collected.

Section B4: Identification of Insect and Plant Samples

Insect samples will be pinned and plant samples will be glued to herbarium sheets and labeled (name of collector, date and location of collection) according to standard procedures. Identification will be made by skilled ARS or Texas A&M entomologists and botanists. Questionable specimens will be made by the ARS Systematic Entomology Laboratory, Beltsville, MD or other recognized taxonomic authorities at Texas A&M University; New Mexico State University, Las Cruces; or University of Texas, Austin, as appropriate. Identified voucher specimens will be preserved by ARS at GSWRL, Temple, TX for comparison and identification of future collections.

Section B5: Quality Control Requirements

The sampling and monitoring conducted in this biological control project involve identification of the insect and plant species and the measurement of insect populations and dispersal, factors causing mortality of the control insect (measurements of parasitism, predation, and competition from exotic leafhoppers and native insects), damage produced on the saltcedar plants (i.e. degree of control), possible effects on non-target plants (not expected to occur), and the recovery of the native plant and animal communities during and after control of saltcedar. Most of this is done by *in situ* counting of the various insects on the plants, categorizing the damage to the plants, and measuring plant size and canopy cover, either in a 10-ha circular area of 40 sentinel saltcedar trees or along transects, and of their nearest neighbors. Normally, the only samples removed from the sample area are of species of uncertain identity that are collected for identification by insect or plant taxonomic specialists, and samples of *Diorhabda* beetle eggs, larvae and adults returned to the Temple laboratory for the rearing of parasites. The area-wide degree of control and of vegetation recovery are also measured by remote sensing.

All these methods are probability based and can be analyzed statistically. They are designed to compare the density, cover and abundance of saltcedar, of native plants and of wildlife both 1) before and after biological control, and 2) in a controlled area with a non-controlled area.

To assure accuracy in the data collection, the field technicians will be taught to identify the different life stages of the beetles by demonstrating the distinguishing characters under a dissecting microscope in the laboratory until they can readily distinguish the *Diorhabda* beetles. Identification will be based on photographs and/or drawings of the identifying characteristics of specimens and by comparisons with identified, pinned *Diorhabda* beetles, or immature stages preserved in vials of alcohol that have been identified by taxonomic specialists. To assure accuracy, the counts made by the field technicians will be compared immediately afterwards by the field biologist by the experienced ARS technicians until the field technician counts are within 10% of the counts made by the experienced ARS personnel at the same time and location.

Laboratory identifications will be made by ARS technicians Robbins and Tracy; all unfamiliar specimens will be sent to taxonomic specialists at the ARS Systematic Entomology Laboratory (SEL), Beltsville, MD; New Mexico State University (NMSU), or Texas A&M University (TAMU), for identification. Voucher specimens will be maintained at the Temple ARS laboratory. Accuracy of the field identification of plants and insects by the ARS technicians (Robbins and Tracy) will be determined by comparison with insects from the same collections (location and date) made by taxonomic specialists at SEL, NMSU, TX A&M, or the University of Texas, Austin.

Initial damage to saltcedar caused by the *Diorhabda* beetles, at low beetle populations especially during the first year of release or within the advancing front of beetle dispersal, will be assessed by placing the tree (or a sample branch) into 5 damage categories. Consistent quality of these estimates is assured by field comparisons and photographs of sample trees with measured damage amounts of 0-10%, 11-30%, 31-70%, 71-95% and 96-100% damage. (See Section B.2.C).

After the first year, and in the central area of high beetle populations, prior experience has shown that almost total defoliation is produced which appears clearly in the remote sensing photography and is simply categorized as "defoliated".

Accuracy of the aereal extent of defoliation will be assured by ground truthing within defoliated areas, along the borders of damage, and in adjacent undamaged areas, made within 3 weeks after the date of the aerial photography. Estimates of resprouting or whole-plant kill will be made the following spring after the plants have resumed growth.

Native plants occurring within the sampling area will be identified by the ARS technician (Robbins). Samples of plants of uncertain identification will be preserved in a standard "plant press", labeled, mounted on standard herbarium mounts, and sent to botanists at TAMU or the University of Texas, Austin for identification. Voucher specimens will be maintained in a herbarium storage cabinet at Temple for future comparison with other samples of these species.

The bird monitoring will be conducted only by highly skilled ornithologists and/or bird watchers of demonstrated proficiency in field identification of birds occurring within the study area (Tom Robbins and/or Kenneth and Joye Johnson). If other identifiers must be used, they will demonstrate their proficiency to within 90% correctness in the field side-by-side with Robbins or the Johnsons. Counts of butterfly species identity and abundance will be performed only by the highly skilled butterfly identifiers (Robbins and Tracy). If other identifiers must be used, only trained entomologists with a course in insect identification will be employed, and they must demonstrate their proficiency to within 90% accuracy in the field side-by-side with Robbins or Tracy. Any unfamiliar species will be collected and sent to taxonomic specialists for identification.

Section B6: Equipment Testing, Inspection, & Maintenance Requirements

Manufacturers' recommendations for scheduling testing, inspection, and maintenance of each piece of equipment will be followed or exceeded. All equipment testing, inspection and maintenance will meet the requirements specified by the EPA. Maintenance and inspection logs will be kept on each piece of field and laboratory equipment; general maintenance checklists will be filled out for sampling equipment prior to each sampling event and serviced as needed. A general maintenance (GM) sheet will be filled out for all sampling equipment during each GM inspection. The GM sheet contains a check list for all equipment and routine maintenance activities (Appendix A). Any equipment, which needs attention, will be serviced during the presampling inspection, with all additional activities described in the comment section. Any maintenance or other required activities that can not be completed during the scheduled GM inspection will be reported to the field supervisor, who then arranges for resolution. The field supervisor checks the presample GM sheets and schedules additional follow-up to ensure that any problems or potential problems are resolved as soon as possible.

To minimize downtime of all measurement systems, all field measurement and sampling equipment, in addition to all laboratory equipment, must be maintained in a working condition. Also, backup batteries or common spare parts will be made available if any piece of equipment fails during use so that repairs or replacement can be made quickly, allowing measurement tasks to be resumed. All staff who use chemicals, reagents, equipment whose parts require periodic replacement and other consumable supplies receive instruction concerning the remaining quantity (unique for each supply) which should prompt a request to order additional supplies.

Equipment used for these experiments consist of HOBO temperature/humidity recorders and HOBO rain gauges located at the field sites. At least once a month, the ARS Project Biologist (Milbrath) or the ARS Project Technician (Tracy or Robbins) will download the data into a notebook computer and examine the equipment for proper functioning. A spare of each type of unit is brought to the field site from GSWRL, Temple and if the installed unit is malfunctioning it is replaced by the spare and the malfunctioning unit is returned to the Temple laboratory for servicing, adjustment or returned to the company for repairs if needed. A log will be maintained at the Temple ARS laboratory of the accuracy checks and the calibration performed.

Section B7: Instrument Calibration and Frequency

All instruments or devices used in obtaining environmental measurement data will be used according to appropriate laboratory or field practices.

All instruments or devices used in obtaining environmental measurement data will be calibrated prior to use. Each instrument has a specialized procedure for calibration and a specific type of standard used to verify calibration. All calibration procedures will meet the requirements specified in the USEPA-approved methods of analysis. The frequency of calibration recommended by the equipment manufacturer, as well as any instructions specified by applicable analytical methods, will be followed. All information concerning calibration will be recorded by the person performing the calibration and will be accessible for verification during either a laboratory or field audit.

All calibration procedures used in the field or laboratory will meet or exceed the calibration frequencies published in the test methods used for this project. Additional calibration procedures may be conducted if laboratory personnel determine additional calibration is warranted as beneficial to this project.

The HOBO temperature recorders and rain gauges will be calibrated at the Temple ARS laboratory annually during the off-season, according to manufacturers directions. Accuracy will be verified monthly in the field. The HOBO temperature recorders will be calibrated by comparison with a certified thermometer and the rain gauges by pouring a measured amount of water into the rain gauge.

The light bar is calibrated by the manufacturer when purchased and each 3 years when returned to the manufacturer for servicing. Accuracy also is compared with a second light bar owned by the Temple ARS laboratory. The light measurements made are relative measurements, being the difference between the measurements under each tree and the measurement immediately thereafter of the open sky beside each tree. Therefore, inaccurate calibration does not affect the accuracy of the relative measurements.

Section B8: Inspection/Acceptance Requirements for Supplies and Consumables

All supplies and consumables received by ARS are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Labels on reagents and chemicals are examined to ensure they are of appropriate quality.

Supplies are inspected by ARS Technicians Tracy or Robbins when received at the ARS Temple laboratory.

Section B9: Data Acquisition Requirements (Non-direct Measurement)

Determinations at sampling sites will be based upon data collected during the time frame of this project. However, data collected from other state or federal projects will be used as supplemental information to meet data quality objectives (see Section A7). In determining biological parameters at sampling sites, data collected prior to this project's initiation will be used to provide some of the pre-infestation data used for pre- and post-benefit comparisons.

The data collected under other projects will be referred to as historical data; this will supplement data from this project in the assessment of changes in vegetation composition, and water conservation.

Data from SC/BC projects at other locations in the western U.S. are used for comparison of *Diorhabda* beetle response and control produced in different climatic/ecological zones.

B10: Data Management

A. Field Collection and Management of Routine Samples

Field staff will visit sampling sites on a weekly to monthly basis to collect data on the control beetles and of their effects on the saltcedar plants. Site identification, date and time, personnel, measurements of field parameters, and any comments concerning weather or conditions at the site are noted on a field data sheet. Field log book and field data sheet is filled out on site for each location visited. An example of a field data sheet is shown in Appendix A.

Specimens of insects and plants are assigned a unique sample identification from a log book, a label with that number is placed on the specimen and recorded on the COC forms, and sent to taxonomic specialists for identification. When the identified specimens are returned, the correct name is recorded on the COC form and the specimens are stored in the plant herbarium or insect collection of ARS at Temple, TX. COCs are kept in three-ring binders in the ARS office for at least five years.

Field data and species identification will be verified by field personnel and/or a data analyst. As field sampling is completed, laboratory personnel will enter the results from laboratory notebooks into EDAS database. The Project Biologist will be responsible for verifying that data in the EDAS database match the data in the laboratory notebooks. After verification has been completed on all data for a group of samples, the laboratory manager will notify the data analyst that a group of data is ready for review. The data analyst will check for abnormalities or problems by examining all field, and laboratory data. Site names, appropriateness of data values, completeness of data, dates and times, container numbers, comments and all other data will be reviewed within the EDAS database. Any questions or abnormalities will be investigated, relying largely on field data and general maintenance sheets, field biologist, laboratory notebooks, and laboratory personnel. As appropriate, corrections will be made to the EDAS database with appropriate documentation maintained.

B. Backup and Disaster Recovery

The electronic data are backed up daily onto an alternate device (i.e. – CD, or comparable media). In the event of a catastrophic systems failure, the media can be used to restore the data. Data generated on the day of the failure may be lost, but can be reproduced from raw data in most cases.

C. Archives and Data Retention

Original data recorded on paper files are stored for at least five years. Data in electronic format are stored on alternate media and/or drives in a climate controlled, fire-resistant storage area at ARS, Temple, TX.

Field data sheets are kept in covered clipboards in the field and transferred to binders each night. These data sheets are placed in categorized and labeled binders at GSWRL—Temple, for 5 years. Each year, as time permits, the data are transferred to an electronic database.

Section C1: Assessments and Response Actions

The commitment to use approved equipment and approved methods when obtaining environmental samples and when producing field or laboratory measurements requires periodic verification that the equipment and methods are, in fact, being employed and being employed properly. This verification will be provided through an annual field and laboratory performance audit performed by the QA officer. Individual field personnel will be observed during the actual field investigation to verify that equipment and procedures are properly applied. Any problems that are discovered in the monitoring procedures that would affect the quality of data collected at the demonstration sites will be addressed by the project participants and followed up with a CAR. Follow-up observations will occur within three months when discrepancies are noted. Also, TSSWCB and EPA will conduct yearly performance audits for this project.

All analyses of field data will have the precision and accuracy of data determined on the particular day that the data were generated.

To minimize downtime of all measurement systems, all field measurement and sampling equipment, and all laboratory equipment must be maintained in a working condition. Also, backup equipment or common spare parts will be available if any piece of equipment fails during use so that repairs or replacement can be made quickly and the measurement tasks resumed.

Section C2: Reports to Management

Quarterly progress reports will note activities conducted in connection with the sampling of field populations of *D. elongata* beetles and their effects on saltcedar and of the plant and wildlife monitoring. Items or areas identified as potential problems , and any variations or supplements to the QAPP will be noted. Corrective action report forms will be utilized when necessary (Appendix A). CARs will be maintained in an accessible location for reference at TFS. CARs that result in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in an update or amendment to the QAPP.

The field measurement and sampling for the project will be done according to the QAPP. However, if the procedures and guidelines established in this QAPP are not successful, corrective action is required to ensure that conditions adverse to quality data are identified promptly and corrected as soon as possible. Corrective actions include identification of root causes of problems and successful correct of identified problem. Corrective Action Reports will be filled out to document the problems and the remedial action taken. Copies of Corrective action reports are included with annual Quality Assurance reports. They will also discuss any problems encountered and solutions made. These QA reports are the responsibility of the Quality Assurance Officer and the cooperating Agency Lead and are available for review upon request.

Section D1: Data Review, Validation and Verification

All data obtained from field and laboratory measurements will be reviewed and verified for integrity and continuity, reasonableness, and conformance to project requirements, and then validated against the data quality objects outlined in Section A7, "Data Quality Objectives for Measurement Data". Only those data that are supported by appropriate quality control data and meet the DQOs defined for this project will be considered acceptable for use.

The procedures for verification and validation of data are described in Section D2, below. The ARS Project Manager is responsible for ensuring that field data are properly reviewed, verified, and submitted in the required format for the project database. The QA officer is responsible for validating that all data collected meet the data quality objectives of the project.

Section D2: Validation and Verification Methods

Quality control aspects of databases include the following:

- Sample data are identified with a unique, sequential sample number.
- Entries into the EDAS database are verified against field data sheets and laboratory notebooks prior to transfer into the EDAS database. This constitutes an on-going internal audit.
- All extreme data outliers will be verified by review of the field data sheets or laboratory notebooks to make sure these points are not transcription errors. If an error is found, the data manager will be notified with the appropriate documentation of the change that is needed in the EDAS databases.
- Unusual circumstances associated with sampling sites or collection of samples are noted in the Comments section of the field notebooks. Comments are copied onto the databases to provide additional information for any questionable results.
- Entries in databases are verified by someone other than the person who enters the data.
- Print-outs of electronically generated data are archived for subsequent verification of data.
- Mistakes in logbooks are crossed out with a single line, corrected, initialed and dated by the person correcting it. This ensures proper lines of communications concerning queries of data validity.

Section D3: Reconciliation with Data Quality Objectives

The ARS Project Manager shall be responsible for reviewing raw data and shall check calculations to verify that data are entered into the database correctly and be responsible for internal error corrections. Corrective Action Reports will be initialed in cases where invalid or incorrect data have been detected.

Data completeness in this project will be relative to the number of sampling events. It will be the goal of this project to achieve 90 percent completeness; however, statistical analysis will be the final indicator of data validity.

Representativeness and comparability of data, while unique to each individual collection site, is the responsibility of the ARS Project Manager. By following the guidelines described in this QAPP, and through careful sampling design, the data collection in this project will be representative of the actual field conditions and comparable to similar applications. The Project Manager will review the final data to ensure that it meets the requirements as described in this QAPP.

Any limitations of use of the data, such as climatic limitations, predation or parasitism of the *Diorhabda* beetles or other limitations, will be discussed in the reports to other users or in published papers.

Section D4: Information Dissemination

Pertinent research and demonstration data will be sent as requested for dissemination to project collaborators; summaries will be presented in the final project report.

Appendix A

<u>Checklist and Forms Used in Data Collections</u>

The following forms are used in the field for collection of data (examples attached).

Form 1. *Diorhabda* Beetle Releases: Insect Populations, Plant Measurements and Damage to Plants
Form 2. *Diorhabda* Beetle Releases: Vegetation Monitoring: Saltcedar and Other Trees – Plant Characteristics & Condition
Form 3. *Diorhabda* Beetle Releases: Vegetation Monitoring Under Saltcedar: 1 m^2 Quadrats by Tree Trunk
Form 4. *Diorhabda* Beetle Releases: Vegetation Monitoring Under Saltcedar: 1 m^2 Quadrats, Canopy Dripline
Form 5. Transects – Plant and Insect Monitoring
Form 6. Bird Monitoring: Breeding Season – Point Counts
Form 7. Bird Monitoring – Winter Area Search
Form 8. Butterfly Monitoring – Transect Survey
Form 9. General Maintenance (GM) Inspection Log

Diorhabda Beetle Releases: Insect Populations, Plant Measurements and Damage to Plants

Tree #: ____ **UTM Zone (NAD 83):** ____ **Easting:** ____ **Northing:** ____ **Date:** ____ **Observer:** ____ **Recorder:** ____ **Photo #** ____

INSECT POPULATIONS, BRANCH MEASUREMENT, DAMAGE

Direction of Marked Primary 40 cm Branch	Green portion of Primary 40 cm Branch Length (cm.)[1,2]	*Diorhabda* % dam	*Diorhabda* no. beetles	*Opsius* % dam	*Opsius* no.	*Chionaspis* % dam	*Chionaspis* no.	Other (List) % dam	Other (List) no.	Number Secondary Twigs[5]	Lengths of 5 Longest Secondary Twigs (cm.)	Total Number of flower Spikelets[6]	Lengths of 5 Longest Spikelets (cm.)	% Damaged? Flower Damage? Yes or No
North			E L A		N A									
East			E L A		N A									
South			E L A		N A									
West			E L A		N A									

Damage to non-target plants by *Diorhabda* beetles (within 5 m of tree canopy)

Plant species	Plant species ht/diam	% dam		No. beetles on plant E,L,A					
North				E L A					
East				E L A					
South				E L A					
West				E L A					

Predator attack on *Diorhabda* beetles

Predator Type	?	No. on branch	No. attacking beetle	
Spider				E L A
Bug				E L A
Ant				E L A
Other				E L A

[1]Average whole tree 1 cm of actively growing stem
[2]Primary branches must be bracketed on both sides with green ties and marked with flagging tape past the base.
[3]Estimate percentages to nearest 10% (*Opsius*=green tamarisk leafhopper, *Chionaspis*=white tamarisk scale)
[4]Estimate number of eggs, larvae or nymphs, and adults
[5]Secondary branches are counted if they measure 1 cm or more in length
[6]Only count flower buds if they show pink or white

Form 1

***Diorhabda* Beetle Releases: Vegetation Monitoring: Saltcedar and Other Trees – Plant Characteristics & Condition Site: ______ Plot: ______**

Tree #: _____ UTM Zone (NAD 83): _______ Easting: ______________ Northing: ______________ Date: ____________ Observer: ______ Recorder: ______ Photo # ______

Tree Height (take 3 measurements 1/3 of the way into the plant canopy)	East) cm	South) cm	West) cm

Canopy Diameter (AT 125 CM HEIGHT)	N-S cm	E-W: cm

DENSIOMETER READING[1]	East) dots	South) dots	West) dots

[1]record number of dots when facing tree; taken 1 m. above ground; 1-2 m. from trunk; same 3 locations as height measurements

REPRODUCTIVE STATUS OF ENTIRE TREE[1]	Flowers :	Buds :	Open Flowers :	Seeds :
	_____Yes	_____Yes	_____Yes	_____Yes
	_____No	_____No	_____No	_____No

[1]check all which apply
Abundant, common, sparce, none (A,C,S)

PHOTON FLUX (LIGHT BAR) READING[1]	East)	South)	West)	In Full Sun)

[1]same locations as height measurements; Lux x 10
10-20/tree

FOLIAGE COLOR[1]	% Green	% Senescing/ Yellow	% Dead Foliage	% Dead Wood	% Regro

[1] record % as follows: N = 0%; L = 1-10%; M = 11-50%; H = 51-95%; T = 96-100%

N=0, L=1-10, ML=11-30, M=31-70, H=71-95, T-%=100%

PERCENT REGROWTH OF ENTIRE TREE[1]	0%	1-10%	11-30%	31-70%	71-90%	91-100%

[1]check which applies

ASSOCIATED WOODY VEGETATION[1]	Distances (cm)	Cardinal Directions	*ASSOCIATED WOODY VEGETATION* (Cont.)	Distances (cm)	Cardinal Directions
****Celtis laevigata* (sugarberry)			+ *Sapindus saponaria* var. *drummondii* (western soapberry)		
***Celtis reticulata* (net-leaf hackberry)			*Salix nigra (black willow)		
****Juniperus pinchotii* (red-berry juniper)			***Tamarix ramosissima *(saltcedar)*		
*Populus deltoides *subsp.* monilifera *(plains cottonwood)*			* Ulmus americana (American elm)		
***Prosopis glandulosa *var.* glandulosa *(honey mesquite)*			Baccharis		

[1] 3 nearest neighbors with diameter at breast height > 2.5 cm (1 in.); *** = abundant w/ saltcedar; ** = common w/ saltcedar; *= occasionally w/ saltcedar; + = w/ saltcedar nr Lake (off site); - = in area and potentially w/ saltcedar; - - = likely in area

NOTES (Plants, Insects, etc.)

Diorhabda Beetle Releases: Vegetation Monitoring Under Saltcedar: 1 m^2 Quadrats by Tree Trunk[a]

Site: _______ Plot: _______Tree #: _______ Date:______________ Observer: _______ Recorder:________Photos_______

SUBSTRATE (e.g., clay, gravel):				
DEPTH OF DUFF/LITTER (CM):				
TOTAL % COVER VEG. > 1 M. HEIGHT				
Woody Veg.:	Herb. Veg:	Dead Wood:	Air:	
TOTAL % COVER ≤ 1 M. HEIGHT				
Woody Veg.:	Herb. Veg:	Duff:	Bare Ground:	
NUMBER OF STEMS & % COVER OF WOODY VEGETATION				

WOODY PLANTS	No. Stems	% Cover[b] ≤ 1 M.	% Cover[b] > 1 M.
TREES			
***Celtis laevigata* (sugarberry)			
Prosopis glandulosa *var.* glandulosa *(honey mesquite)*			
Sapindus saponaria *var.* drummondii *(western soapberry)*			
***Tamarix ramosissima *(saltcedar)*			
*Ulmus americana *(American elm)*			
SHRUBS			
*Lycium berlandieri *(Berlandier wolfberry)*			
** Prunus angustifolia *(chickasaw plum)*			
SUBSHRUBS			
***Chloracantha spinosa* (spiny aster)			
WOODY VINES			
** *Ibervillea lindheimeri* (balsam-gourd)			
** *Cocculus carolinus* (Carolina snailseed)			
** *Smilex bona-nox* (catbrier)			

% COVER OF HERBACEOUS VEGETATION — HERBS	% Cover[c] ≤ 1 M.	% Cover[c] > 1 M.
GRASSES – Perrenial		
Rhizomatous & Stoloniferous Grasses		
* ***Bouteloua curtipendula* var. *curtipendula*** (side-oats gramma)		
** ***Cynodon dactylon*** (bermuda grass)		
* ***Distichlis spicata*** (salt grass)		
- ***Hilaria mutica*** (tobosa)		
****Panicum obtusum*** (vine-mesquite)		
*Pascpopyrum smithii (western wheat grass)		
*Poa arachnifera (Texas blue grass)		
***Poa pratensis (Kentucky blue grass)		
*** Sorghum halepense (Johnson grass)		
Bunch Grasses (weakly rhizomatous or w. stoloniferous)		
* ***Aristida purpurea* var. *longiseta*** (red threeawn)		
* ***Bothriochloa laguroides* subsp. *torreyana*** (silver bluestem)		
** ***Elymus canadensis*** (Canada wild rye)		
*** ***Elymus virginicus*** (Virginia wild rye)		
** ***Nassella leucotricha*** (Texas winter grass)		
* ***Panicum antidotale*** (blue panic)		
****Panicum rigidulum*** (red-top panic)		
** ***Panicum virgatum*** –lowland gr. form (lowland switch grass)		
****Tridens albescens*** (white tridens)		

% COVER OF HERBACEOUS VEGETATION — *GRASSES – Perrenial*	% Cover[c] ≤ 1 M.	% Cover[c] > 1 M.
Bunch Grasses (cont.)		
** ***Setaria leucophila*** (plains bristlegrass)		
*** Sporobolus compositus *var.* compositus *(tall dropseed)*		
* Sporobolus cryptandrus *(sand dropseed)*		
GRASSES - Annual		
*** Bromus catharticus (rescue grass)		
***Bromus japonicus *(Japanese brome)*		
***Bromus tectorum *(downy brome)*		
* ***Echinochloa crus-galli*** (barnyard grass)		
** Hordeum pusillum (little barley)		
* Lolium temulentum (darnel)		
SEDGES & RUSHES		
***Carex tetrastachya (four-angle caric sedge)		
+ Cyperus esculentus (yellow nut-grass)		
+ ***Eleocharis palustris*** (large-spike spike-rush)		
BROADLEAVES - Perrenial		
** ***Artemesia ludoviciana* subsp. *mexicana*** (Mexican sagebrush)		
* ***Clematis texensis*** (scarlet clematis)		
** ***Cynanchum laeve*** (bluevine)		
* ***Rumex altissimus*** (smooth dock)		
** ***Rumex crispus*** (curly dock)		
** ***Solanum eleagnifolium*** (silver-leaf nightshade)		

% COVER OF HERBACEOUS VEGETATION	% Cover[c] ≤ 1 M.	% Cover[c] > 1 M.
BROADLEAVES -Perrenial *(cont.)*		
****Solidago canadensis*** (common goldenrod)		
** ***Symphiotrichum divaricatum*** (blackweed)		
* ***Symphiotrichum ericoides*** (heath aster)		
BROADLEAVES - Annual		
*** Ambrosia psilostachya (western ragweed)		
****Ambrosia trifida* var. *texana*** (giant ragweed)		
*** ***Conyza canadensis*** (hosrseweed)		
* ***Cyclospermum leptophyllum*** (slim-lobe celery)		
** ***Gutierrezia dracunculoides*** (common broomweed)		
* Helianthus anuus (common sunflower)		
* ***Iva annua*** (marsh-elder)		
* ***Kochia scoparia*** (kochia)		
** ***Lepidium densiflorum*** (prairie pepperweed)		
* ***Physalis angulata*** (cut-leaf ground cherry)		
** ***Plantago virginica*** (dwarf plantain)		
****Salsola tragus*** (Russian thistle)		
*** ***Sonchus oleraceus*** (common sow-thistle)		
*** ***Torilis arvensis*** (hedge-parsley)		
* ***Xanthium strumarium* var. *canadense*** (cocklebur)		

NOTES:

Form 3

[a] 0.05 m N of SC trunk; [b] Divide for same plant by height; [c] of total vegetation < 1 M. height; recording % is optional, at least mark if present; *** = abundant w/ saltcedar; ** = common w/ saltcedar; *= occasionally w/ saltcedar; + = w/ saltcedar or Lake (off site); - = in area and potentially w/ saltcedar; - - = likely in area.

Diorhabda Beetle Releases: Vegetation Monitoring Under Saltcedar: 1 m^2 Quadrats, Canopy Dripline[a]

Site: _______ Plot: _______ Tree #: _______ Date: _______________ Observer: _______ Recorder: ________ Photos _______

SUBSTRATE (e.g., clay, gravel):			
DEPTH OF DUFF/LITTER (CM):			
TOTAL % COVER VEG. > 1 M. HEIGHT			
Woody Veg.:	Herb. Veg:	Dead Wood:	Air:
TOTAL % COVER ≤ 1 M. HEIGHT			
Woody Veg.:	Herb. Veg:	Duff:	Bare Ground:
NUMBER OF STEMS & % COVER OF WOODY VEGETATION			

		% Cover[b]	
WOODY PLANTS	No. Stems	≤ 1 M.	> 1 M.
TREES			
***Celtis laevigata* (sugarberry)			
Prosopis glandulosa *var.* glandulosa *(honey mesquite)*			
Sapindus saponaria *var.* drummondii *(western soapberry)*			
***Tamarix ramosissima *(saltcedar)*			
*Ulmus americana *(American elm)*			
SHRUBS			
*Lycium berlandieri *(Berlandier wolfberry)*			
** Prunus angustifolia *(chickasaw plum)*			
SUBSHRUBS			
****Chloracantha spinosa* (spiny aster)			
WOODY VINES			
** *Ibervillea lindheimeri* (balsam-gourd)			
** *Cocculus carolinus* (Carolina snailseed)			
** *Smilex bona-nox* (catbrier)			

Form 4

% COVER OF HERBACEOUS VEGETATION	% Cover[c]	
HERBS	≤ 1 M.	> 1 M.
GRASSES – Perrenial		
Rhizomatous & Stoloniferous Grasses		
* *Bouteloua curtipendula* var. *curtipendula* (side-oats gramma)		
** *Cynodon dactylon* (bermuda grass)		
* *Distichlis spicata* (salt grass)		
- *Hilaria mutica* (tobosa)		
****Panicum obtusum* (vine-mesquite)		
*Pascopyrum smithii (western wheat grass)		
*Poa arachnifera (Texas blue grass)		
***Poa pratensis (Kentucky blue grass)		
*** Sorghum halepense (Johnson grass)		
Bunch Grasses (weakly rhizomatous or w. stoloniferous)		
* *Aristida purpurea* var. *longiseta* (red threeawn)		
* *Bothriochloa laguroides* subsp. *torreyana* (silver bluestem)		
** *Elymus canadensis* (Canada wild rye)		
*** *Elymus virginicus* (Virginia wild rye)		
** *Nassella leucotricha* (Texas winter grass)		
* *Panicum antidotale* (blue panic)		
**Panicum rigidulum* (red-top panic)		
** *Panicum virgatum* –lowland gr. form (lowland switch grass)		
**Tridens albescens* (white tridens)		

% COVER OF HERBACEOUS VEGETATION	% Cover[c]	
GRASSES – Perrenial		
Bunch Grasses (cont.)	≤ 1 M.	> 1 M.
** *Setaria leucophila* (plains bristlegrass)		
*** Sporobolus compositus *var.* compositus *(tall dropseed)*		
* Sporobolus cryptandrus *(sand dropseed)*		
GRASSES - Annual		
*** Bromus catharticus (rescue grass)		
***Bromus japonicus *(Japanese brome)*		
***Bromus tectorum *(downy brome)*		
* *Echinochloa crus-galli* (barnyard grass)		
** Hordeum pusillum (little barley)		
* Lolium temulentum (darnel)		
SEDGES & RUSHES		
***Carex tetrastachya (four-angle caric sedge)		
+ Cyperus esculentus (yellow nut-grass)		
+ *Eleocharis palustris* (large-spike spike-rush)		
BROADLEAVES - Perrenial		
** *Artemesia ludoviciana* subsp. *mexicana* (Mexican sagebrush)		
* *Clematis texensis* (scarlet clematis)		
** *Cynanchum laeve* (bluevine)		
* *Rumex altissimus* (smooth dock)		
** *Rumex crispus* (curly dock)		
** *Solanum eleagnifolium* (silver-leaf nightshade)		

% COVER OF HERBACEOUS VEGETATION	% Cover[c]	
BROADLEAVES -Perrenial *(cont.)*	≤ 1 M.	> 1 M.
***Solidago canadensis* (common goldenrod)		
** *Symphiotrichum divaricatum* (blackweed)		
* *Symphiotrichum ericoides* (heath aster)		
BROADLEAVES - Annual		
*** Ambrosia psilostachya (western ragweed)		
**Ambrosia trifida* var. *texana* (giant ragweed)		
*** *Conyza canadensis* (hosrseweed)		
* *Cyclospermum leptophyllum* (slim-lobe celery)		
** *Gutierrezia dracunculoides* (common broomweed)		
* Helianthus anuus (common sunflower)		
* *Iva annua* (marsh-elder)		
* *Kochia scoparia* (kochia)		
** *Lepidium densiflorum* (prairie pepperweed)		
* *Physalis angulata* (cut-leaf ground cherry)		
** *Plantago virginica* (dwarf plantain)		
**Salsola tragus* (Russian thistle)		
*** *Sonchus oleraceus* (common sow-thistle)		
*** *Torilis arvensis* (hedge-parsley)		
* *Xanthium strumarium* var. *canadense* (cocklebur)		

NOTES:

[a] 0.05 m N of SC trunk; [b] Divide for same plant by height; [c] of total vegetation < 1 M. height; recording % is optional, at least mark if present; *** = abundant w/ saltcedar; ** = common w/ saltcedar; *= occasionally w/ saltcedar; + = w/ saltcedar nr Lake (off site); - = in area and potentially w/ saltcedar; - - = likely in area.

Transects: Plant and Insect Monitoring

Transect #________, Direction from origin __________						
				Diorhabda on SC		
Distance along Transect	Plant sp.	Plant ht/diam	Info on SC (most info from 10 ha circle form)	% dam	No. beetles	Damage non-target plants

BIRD MONITORING: BREEDING SEASON

POINT COUNT DATA FORM

Observer____________________

Page__of __pages

_ _ STATE | _ _ _ _ _ _ _ _ REGION | _ _ _ _ _ STATION | _ _ MONTH | _ _ DAY | _ _ _ _ YEAR | _ _ VISIT NO.

			0 - 3 minutes			3 - 5 minutes	
PT #	TIME	SPECIES	<50 M	>50 M	Fly over	<50 M	>50 M	Fly-overs

Form 6

BIRD MONITORING: BREEDING SEASON

WINTER AREA SEARCH

Observer____________________________

Page___of ___pages

_ _ STATE   _ _ _ _ _ _ _ _ REGION   _ _ _ _ _ _ STATION   _ _ MONTH   _ _ DAY   _ _ _ _ YEAR   _ _ VISIT NO.

WEATHER:

PLOT #		TIME				SPECIES				GROUD	HERB	SHRUB	TREE	OUTSIDE	NOTES

Form 7

Butterfly Monitoring: Transect Survey

Location:	Transect No.:	Habitat:	
	Name of surveyor:	Date:	Temperature and Weather:

Time	Survey Activity (4)		Location (Optional)	Common Name of Butterfly Seen	Numbers and Behavior of Butterfly (4)			Habitat Use of Butterfly	
	Start	Stop			Resting	Flying	Feeding	Resting Substrate	Food Source

Form 8

General Maintenance (GM) Inspection Log

HOBO-SN# ______________________________

Location	Date inspected	Maintenance performed

Form 9

Separate Sheets for each item of equipment

HOBO
Rain gauges
Light bar (borrowed)
Weather station (not bought yet)